Tinnitus

An MRI Study on Brain Mechanisms

RIJKSUNIVERSITEIT GRONINGEN

Tinnitus

An MRI Study on Brain Mechanisms

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“Η αληθινή σοφία έρχεται στον καθένα μας όταν συνειδητοποιούμε πόσο λίγα γνωρίζουμε για τη ζωή, τον εαυτό μας και τον κόσμο γύρω μας.”

“True wisdom comes to each of us when we realize how little we understand about life, ourselves, and the world around us.”

Socrates (470-399 B.C.)

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The cover is designed by Kaat Boyen and expresses the desperation of tinnitus patients.

Table of Contents

Chapter 1

Introduction to the Thesis

The common thread throughout this thesis is tinnitus. The first part of the thesis is intended as a general introduction to this phenomenon and to the main concepts and techniques associated with it. It provides an overview of the possible causes and consequences of tinnitus (**section 1.1**). Since tinnitus is often associated with hearing loss, the auditory pathway is described (**section 1.2**) and causes of hearing loss and the effect of hearing loss on the central auditory system are explained (**section 1.3**). Finally, **section 1.4** gives a brief overview of the methods we used in the studies that are described in this thesis.

1.1 Tinnitus

Tinnitus, or 'ringing in the ears' in common language, is the percept of sound in the absence of any external sound. Although the experience of short bursts of noise is almost universal, tinnitus is typically defined as noise that lasts at least for five minutes (Davis, 1995). Tinnitus is described as subjective or objective. Differentiating between both types of tinnitus is important for a successful approach to the tinnitus patient. Objective tinnitus is a sound that can be heard by the patient as well as by a clinician. Thus, this kind of tinnitus can be 'objectified' by others. Sources of objective tinnitus are usually of vascular or muscular origin (Chandler, 1983; Weissman and Hirsch, 2000; Howsam et al. 2005; Liyanage et al., 2006; Sonmez et al., 2007). Since sound from the body leads to an auditory percept via normal hearing mechanisms, objective tinnitus is typically not caused by an affected hearing organ. Objective tinnitus is rare and is mainly described as case reports.

Subjective tinnitus can be heard only by the sufferer. There is no acoustical source and this kind of tinnitus is a 'subjective' phantom percept. The type of perceived sound mostly resembles one or more tones or noises and can be perceived unilaterally, bilaterally or centrally in the head. Prevalence estimates generally range from 7 to 20% (Hoffman and Reed, 2004). Tinnitus is less prevalent in women than in men. Furthermore, its prevalence increases with age (Lockwood et al., 2002; see **Figure 1.1**). Approximately 40% of the tinnitus patients also suffer from hyperacusis, a diminished tolerance to sound (Baguley, 2003). This thesis deals with subjective tinnitus only, and for the remainder of this text we will drop the adjective "subjective".

The consequences of tinnitus may be mild but may also have a devastating impact on the ability to function in daily life (Lockwood et al., 2002). Most patients with chronic tinnitus are continuously aware of the tinnitus percept, but are able to cope effectively with the disturbance. However, for some patients the tinnitus is more than a trivial annoyance, resulting in feelings of desperation and even suicidal thoughts (Dobie, 2003). A variety of additional symptoms is often reported, including stress, anxiety, depression, insomnia and irritability (Møller, 2000; Hébert and Lupien, 2007; Langguth et al., 2011).

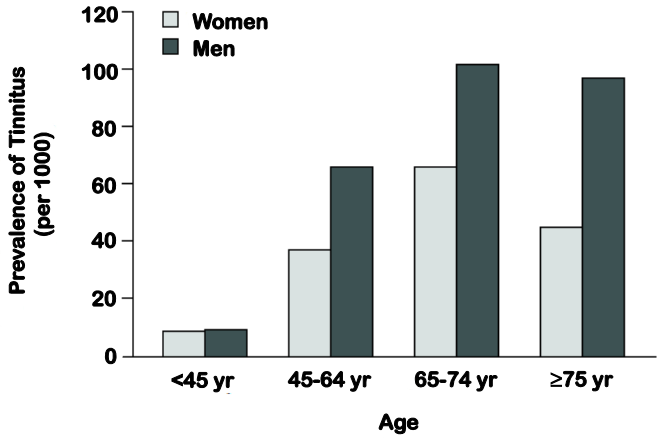


Figure 1.1. The prevalence of tinnitus. Adapted from Lockwood et al. (2002).

The underlying pathophysiology of tinnitus is still poorly understood. Aging or loud-noise exposure, both of which lead to some form of hearing loss, are often associated with tinnitus. Since the accompanying hearing loss usually has a peripheral origin, the generator of tinnitus initially was thought to lie in the inner ear. However, dissection of the auditory nerve does not eliminate the tinnitus in the majority of subjects (House and Brackmann, 1981; Berliner et al., 1992), proving that mechanisms in the central auditory system must play an important role in the generation of tinnitus.

Several neurophysiological studies in animals suggest that tinnitus arises from reorganization that involves a disruption of the normal balance between excitation and inhibition in the central nervous system. A compelling hypothesis is that tinnitus results from a down-regulation of inhibitory neurotransmission in the central auditory pathway (see Roberts et al., 2010 for a review). This loss of inhibition may be a compensatory response to loss of afferent input from the ear to the brain, such as that caused by age-related hearing loss, the most common form of hearing impairment in humans. Compensatory plastic changes in response to peripheral hearing loss may result in the pathologic neural activity that underpins tinnitus. It has been hypothesized that the neural correlates of tinnitus include increased spontaneous neural activity and changes in neural synchrony (Noreña and Eggermont, 2003; Seki and Eggermont, 2003).

In summary, tinnitus is a phantom sound in the sense that it does not correspond to an acoustic source that can be detected by others. The consequences of this sound percept may be mild, but it may also lead to severe additional complaints. Tinnitus may be caused by central adaptation in the brain to altered peripheral input due to hearing loss.

1.2 The auditory pathway

1.2.1 Peripheral auditory system

The function of the ear is to transduce acoustic vibrations in the ear into neural signals for the brain where these sounds can be interpreted. The ear is divided into three parts: the outer ear, the middle ear and the inner ear (**Figure 1.2**).

The *outer ear* includes the auricle or pinna and the auditory canal. The border with the middle ear is formed by the eardrum, also known as the tympanic membrane. Vibrations in the air travel through the ear canal to the tympanic membrane. Once the sound vibrations reach the tympanic membrane, they enter the middle ear.

In the *middle ear*, the ossicles (from the Latin for "little bones") transfer movements of the tympanic membrane into movements of the oval window that forms the connection between the middle ear and the inner ear.

The sound wave now enters the cochlea of the *inner ear*. The cochlea converts the entered sound vibrations into electrical pulses (‘action potentials’), which leave the cochlea via the auditory nerve. This transduction from mechanical energy to electrochemical energy occurs in a structure located in the cochlea, the organ of Corti, which contains rods of Corti, various supporting cells and receptor cells. These receptor cells are called hair cells and form synapses with neurons. The sound wave deflects the tiny hair bundles on these hair cells leading to modulation of the intracellular potential. The electrical potential changes cause the generation of action potentials in the auditory nerve fibers.

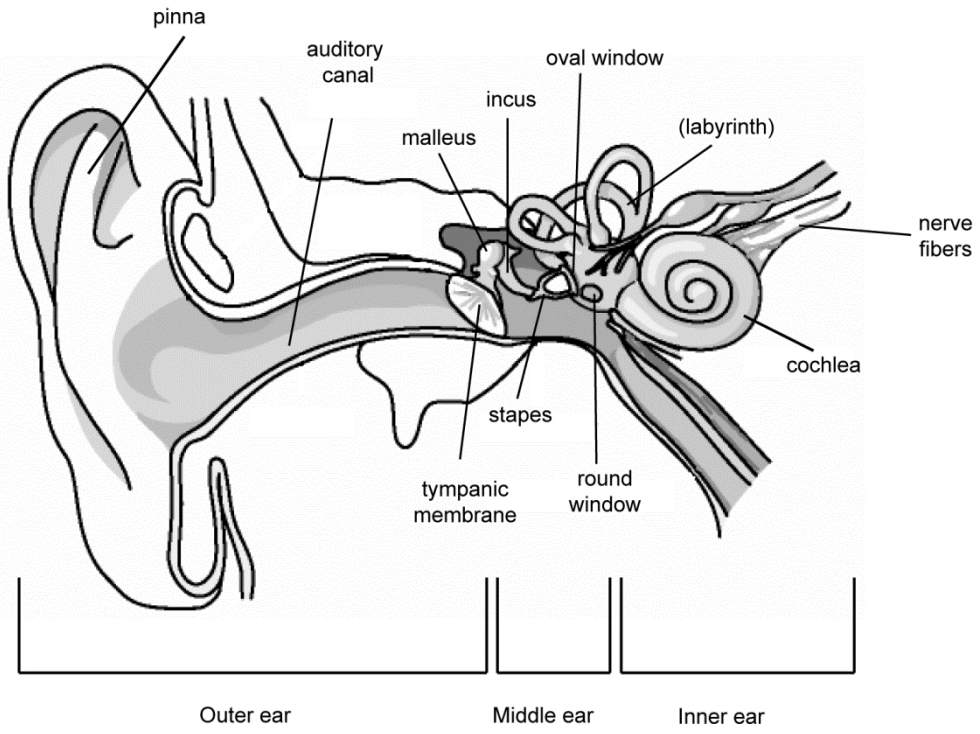


Figure 1.2. The human peripheral ear consists of three parts: the outer ear (from pinna to tympanic membrane), the middle ear (from tympanic membrane to oval window) and the inner ear (cochlea).

1.2.2 Central auditory system

Action potentials in the auditory nerve excite a complex network of auditory areas in the brain. This pathway is schematically illustrated in **Figure 1.3**. This path is known as the principal ascending auditory pathway.

The fibers of the auditory nerve enter the brain in the lower brainstem via the *cochlear nucleus* (CN), ipsilateral to the cochlea from where the axons originate. This region is anatomically and physiologically divided into the dorsal cochlear nucleus (DCN), the anterior ventral cochlear nucleus (AVCN) and posterior ventral cochlear nucleus (PVCN).

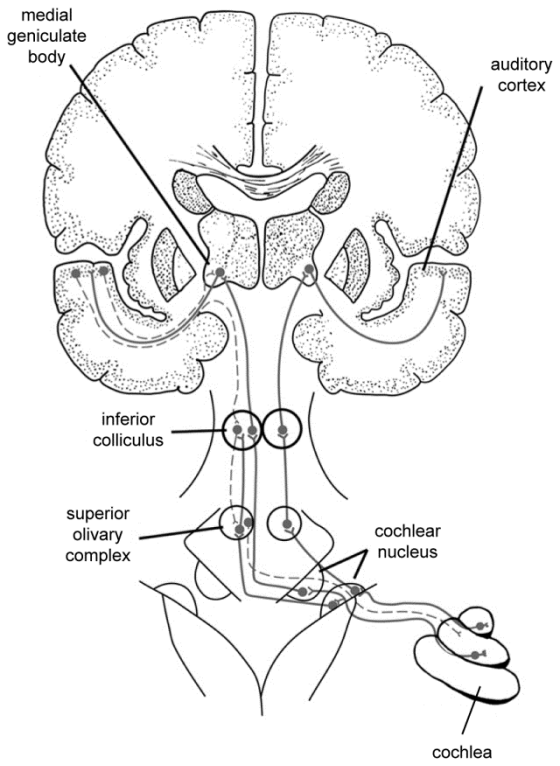


Figure 1.3. Schematic overview of the central auditory pathway. Action potentials in the auditory nerve are sent to the ipsilateral cochlear nucleus. In succession, fibers mainly project to the contralateral superior olivary complex, inferior colliculus, medial geniculate body and finally the auditory cortex.

Subsequently, the auditory pathway continues through the *superior olivary complex* (SOC). The SOC is located in the pons and receives predominantly projections from the contralateral VCN.

From the SOC and via the lateral lemniscus, axons are sent out to the *inferior colliculus* (IC) in the midbrain. The IC is an auditory nucleus consisting of three parts: the central nucleus, the external nucleus and the pericentral nucleus. The IC does not only receive input from the CN via the SOC, but also directly from the CN.

From the IC, axons project to the *medial geniculate body* (MGB) of the auditory thalamus, which represents the thalamic relay of auditory signals between the IC and auditory cortex.

Finally, the axons from the thalamus project to the *auditory cortex* (AC), located in the upper part of the temporal lobe. Based on cell types (i.e. cytoarchitecture), the AC can be divided in several areas (Brodmann, 1909). It can be divided in Brodmann area (BA) 41, BA 42 and BA 22. BA 41 roughly overlaps with the primary auditory cortex. BA 42 is located next to BA 41 and plays a role as secondary auditory cortex. BA 22 surrounds these areas and is also known as the auditory association cortex. Probably, the primary auditory cortex performs processing of basic sound characteristics like frequency and intensity level analysis, whereas non-primary areas may be involved in spectrotemporally more complex sounds (Hall et al., 2001; Hall et al., 2003; Langers et al., 2003).

1.3 Hearing impairment and its effects on the central auditory system

Hearing loss is the most common age-related sensory disorder (Gates and Mills, 2005; Liu and Yan, 2007). Its prevalence increases with age (Lockwood et al., 2002; see **Figure 1.4**), as is the case for tinnitus. This underscores the close relation between tinnitus and hearing loss.

A defect anywhere in the auditory pathway induces hearing loss or deafness. Types of hearing loss are determined by the nature of the organic defect. A main distinction is made between conductive and sensorineural hearing loss. Conductive hearing loss is characterized by a defect in the outer ear or, more commonly, the middle ear. In contrast, for sensorineural hearing loss, the defect is located in the inner ear, auditory nerve or in the brain. The most common form of sensorineural hearing loss is presbyacusis, or age-related hearing loss. Correspondingly, the prevalence of hearing systematically increases with age (see **Figure 1.4**). In some patients, both a conductive and a sensorineural component is seen, which is referred to as a mixed hearing loss.

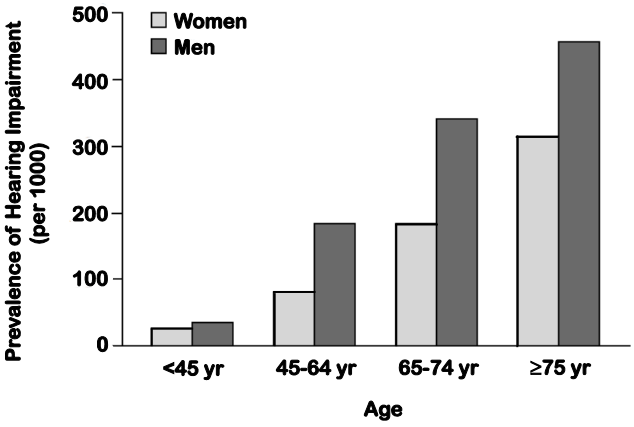


Figure 1.4. The prevalence of hearing loss. Adapted from Lockwood et al. (2002).

The central auditory system is known to potentially display changes in its function and structure as a result of prolonged changes in neural input from the ear, for instance due to various types of hearing disorders. Such plastic changes presumably play a role in the development of tinnitus (Rauschecker, 1999; Noreña and Eggermont, 2003; Seki and Eggermont, 2003; Rauschecker et al., 2010). In animal studies, it is shown that sensorineural hearing loss caused by ototoxic drugs coincides with decreased activation to sound in bilateral primary auditory cortices (Zhang et al., 2006). Restricted cochlear lesions in neonatal, juvenile and adult cats result in reorganization of the primary auditory cortex (Harrison et al., 1991; Rajan et al., 1993; Eggermont and Komiya, 2000; Rajan and Irvine, 2010). Furthermore, cochlear damage caused by noise exposure is associated with structural changes in the mouse cochlear nucleus and inferior colliculus (Coordes et al., 2012). Although the results vary between studies, the consequences of peripheral hearing loss appear to extend to cortical morphology in humans as well (Husain et al., 2011; Boyen et al., 2012; Eckert et al., 2012). Together, the outcomes of these studies show that hearing loss leads to extensive structural and functional changes in the brain.

1.4 Methods

In all experiments magnetic resonance imaging (MRI) scans were acquired from the participating subjects. Two main types of MRI scans exist. During acquiring *functional* MRI scans, the subjects perform a specific task. Basically, the function of the brain related to the task is measured. *Structural* brain imaging deals with the structure of the brain. Acquiring *structural* MRI brain images implies that a high-resolution scan is acquired when the subject does not perform a task, is relaxed and keeps the eyes open. Functional and structural MRI offer the possibility to examine the human central auditory system non-invasively. In the following paragraphs, both acquisition methods will be briefly explained.

1.4.1 Measuring brain activity

A basic approach to our research is to search for abnormal brain activity that may account for tinnitus. One method to measure brain activity is fMRI. Functional MRI relies on the fact that local brain activity results in a local increase in blood flow, which is known as the blood oxygenation level dependence (BOLD) response. This local effect causes a small change of the magnetic properties of brain tissue that can be detected and localized by an MRI scanner. Thus, fMRI is an indirect method to measure brain activity (Jezzard et al., 2001).

Figure 1.5 shows the temporal characteristics of the BOLD response to a brief stimulus. As is shown, the response peaks at 4-6 seconds after the stimulus onset. This turns out to be very useful in auditory fMRI, where a so-called sparse imaging protocol has been used to measure brain responses (see *chapters 4* and *6*).

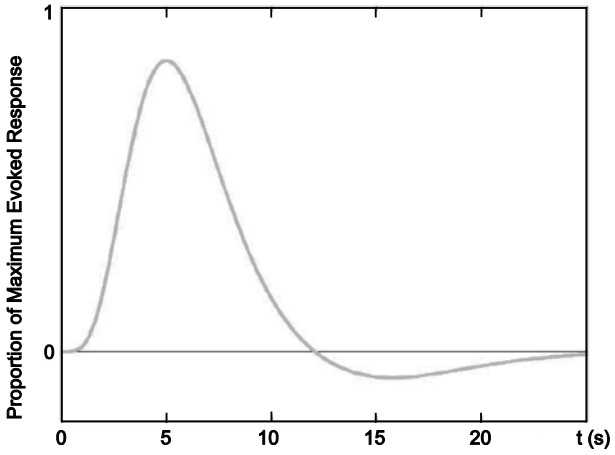


Figure 1.5. Schematic representation of the blood oxygenation level dependence (BOLD) response. This response is characterized with an onset delay, a peak and a post-stimulus undershoot before rising back to the pre-stimulation baseline level.

1.4.2 Measuring structural differences in the brain

Functional changes or properties of the brain are known to be related to structural properties (Maguire et al., 2000). We were interested in structural characteristics of the brain associated with tinnitus. Here, the basic paradigm is a comparison between subjects with and without tinnitus. One method to make such a comparison is voxel-based morphometry (VBM; Good et al., 2001; Ashburner and Friston, 2000). VBM involves the analysis of high-resolution anatomical MRI scans. Structural differences between subjects with and without tinnitus, respectively, are assumed to be related to small differences in the MRI signal of gray matter in the brain. The comparison is made between corresponding voxels (i.e. the 3-D equivalent of 2-D pixels in a photograph) in the brain of the two subjects groups, respectively. Hence the term voxel-based morphometry.

1.5 Aim and outline of the thesis

1.5.1 Aim

This thesis describes a number of magnetic resonance imaging (MRI) studies of the central auditory system in the human brain. The aim of this research project is to gain more insight into the functional and structural changes associated with tinnitus.

To achieve this aim, a number of MRI studies was designed and performed. The obtained results of these studies are presented in this work.

1.5.2 Outline

The thesis is divided into two main parts.

PART A. TINNITUS ACCOMPANIED BY MILD TO MODERATE HEARING IMPAIRMENT

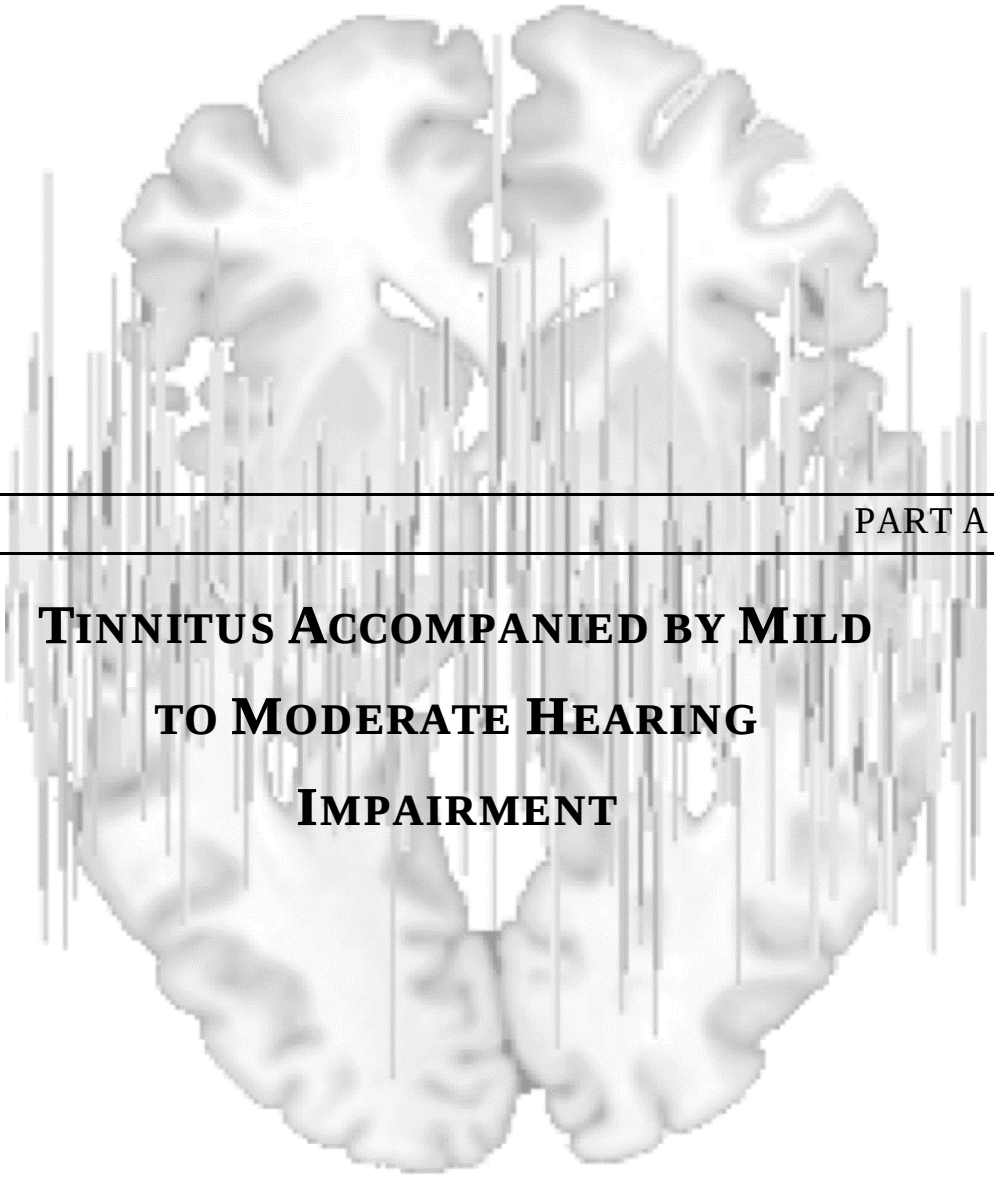
This part describes a comprehensive study of tinnitus in patients with mild to moderate sensorineural hearing loss and contains three chapters. The first chapter describes the characteristics of the hearing-impaired subjects who were included in our analysis. The second chapter of part A describes a VBM analysis of structural brain differences associated with tinnitus. The third and final chapter of part A describes a functional MRI study of the correspondence between brain activity and tinnitus. Together, these chapters provide a comprehensive insight in structural and functional characteristics associated with tinnitus.

PART B. SOMATIC TINNITUS: THE ABILITY TO EVOKE OR MODULATE TINNITUS BY BODILY MANEUVERS

Part B contains two chapters. Some tinnitus patients are able to modulate their tinnitus by somatic maneuvers (e.g. jaw clenching or eye movements). This ability offers unique opportunities to study the relation between the tinnitus percept and brain activity by functional neuroimaging. The first chapter of part B provides an introduction to the phenomenon of somatic tinnitus by means of an overview of brain imaging studies. The second chapter describes a neuroimaging study in

patients who can modulate their tinnitus by lateral gaze of their eyes. The psychoacoustic tinnitus characteristics and the related brain activity in patients with gaze-evoked tinnitus are described.

The thesis is concluded with a general discussion in which the main results of the different studies will be integrated in an effort to better understand the tinnitus phenomenon.



PART A

TINNITUS ACCOMPANIED BY MILD TO MODERATE HEARING IMPAIRMENT

Chapter 2

Characteristics of the Hearing-Impaired Subjects Participating in the Studies of Part A

In the following MRI studies (*chapters 3* and *4*), hearing-impaired subjects were enrolled. The participants took part in an extensive test battery consisting of questionnaires and psychoacoustic tests. The purpose of this introductory section is to provide a description of the subjects' characteristics that were assessed by the test battery.

2.1 Materials and methods

2.1.1 Subjects

All patients were recruited at the University Medical Center Groningen (UMCG) and at hearing aid dispensers located in Groningen, The Netherlands. The first group included hearing-impaired subjects who suffered from tinnitus (HI+T group). The second group included hearing-impaired subjects without tinnitus (HI group). Pure-tone audiometry was performed with a clinical audiometer using six octave frequencies (0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 kHz). In order to be enrolled in the study, the subjects had to meet the following inclusion criterion: Pure-tone average (PTA) air conduction hearing thresholds at the octave frequencies 1.0, 2.0 and 4.0 kHz should $30 \leq PTA \leq 60$ dB in both ears.

Handedness was assessed by means of a translated version of the Edinburgh Inventory (Oldfield, 1971), completed by all subjects.

In the tinnitus group, the subjectively perceived loudness was recorded on a numeric rating scale from 0 (tinnitus not audible at the time) to 10 (tinnitus sounds as loud as imaginable). In order to verify whether the scanning session, in which the participant took part, influenced the tinnitus loudness, this tinnitus loudness rating was performed before and after the scanning session.

2.1.2 Questionnaires

All Subjects

All subjects completed a *Hospital Anxiety and Depression Scale* (HADS) questionnaire, in order to screen for possible anxiety and depression. The HADS has been developed to be a reliable instrument for detecting states of depression and anxiety in the setting of an outpatient clinic (Zigmond and Snaith, 1983). The HADS is a self-report measure consisting of two subscales, a seven-item anxiety and a seven-item depression scale. The questions in both scales are answered on a 4-point Likert scale from 0-3 with a score range of 0-21 for each scale. Answer possibilities depend on the question. For example, answer possibilities to the first question “I feel tense” are ‘not at all’, ‘from time to time’, ‘a lot of the time’ and ‘most of the time’. Both scales require that patients describe how they have been feeling in the past week. Higher total scores represent a larger likeliness of anxiety or depression. Scores up to and including seven exclude anxiety or depression. A

score of 8-10 is considered to indicate a possible anxiety or depression. A score of 11-21 is indicative of a probable anxiety or depression.

In order to assess the presence of hyperacusis, a translated version of the *Hyperacusis Questionnaire* (HQ; Khalfa et al., 2002) was administered to all participating subjects. Vernon (1987) defined hyperacusis as 'an unusual tolerance to ordinary environmental sounds'. This definition implies that patients suffering from hyperacusis report discomfort to sounds that are acceptable for most normal-hearing persons. The 14-item HQ consists of questions examining the attentional (4 items), social (6 items) and emotional (4 items) dimensions of hyperacusis. Answers to each question are given on a 4-point Likert scale ranging from 'no' (0 points), 'yes, a little' (1 point), 'yes, quite a lot' (2 points) to 'yes, a lot' (3 points), resulting in total scores ranging from 0-42. A total score greater than 28 represents a stronger auditory hypersensitivity.

Tinnitus Subjects

In the tinnitus subjects, tinnitus severity was measured by three validated questionnaires that we used in a Dutch translation: the Tinnitus Handicap Inventory, the Tinnitus Reaction Questionnaire and the Tinnitus Coping Style Questionnaire.

The *Tinnitus Handicap Inventory* (THI) is a self-reported tinnitus handicap questionnaire (Newman et al., 1996). The 25-item THI consists of questions examining the functional (11 items), emotional (9 items) and catastrophic reactions (5 items) to tinnitus. Answers to each question are given on a 3-point Likert scale ranging from 'no' (0 points), 'sometimes' (2 points) to 'yes' (4 points), resulting in a total score in the range 0-100. Higher total scores represent a higher impact of the tinnitus on everyday life. Reference scores ranging from 0-16 indicate no handicap, 18-36 indicate mild handicap, 38-56 indicate moderate handicap, and 58-100 indicate severe handicap.

The second questionnaire is the *Tinnitus Reaction Questionnaire* (TRQ), which was developed to measure tinnitus-related psychological stress (Wilson et al., 1991). The 26-item TRQ consists of questions primarily based on the symptom categories described by Tyler and Baker (1983). Answers to each question are given on a 5-point Likert scale ranging from 'not at all' (0 points), 'a little of the time' (1 point), 'some of the time' (2 points), 'a good deal of the time' (3 points) to 'almost all of the time' (4 points), resulting in a total score in the range 0-104. Higher total scores represent greater psychological distress caused by tinnitus.

Finally, the *Tinnitus Coping Style Questionnaire* (TCSQ) was developed to assess the frequency with which tinnitus sufferers apply coping style strategies to adapt to their tinnitus (Budd and Pugh, 1996). The TCSQ can be divided into two subscales, each consisting of 20 items: $TCSQ_{mal}$ and $TCSQ_{effect}$, referring to a specific coping style. The $TCSQ_{mal}$ involves the use of a broad range of maladaptive coping strategies. On the other hand, the $TCSQ_{effect}$ involves the use of a broad range of effective coping strategies. Answers to each question are given on a 7-point Likert scale ranging from 'never' (1 point), 'rarely' (2 points), 'occasionally' (3 points), 'sometimes' (4 points), 'often' (5 points), 'nearly always' (6 points) and 'always' (7 points), resulting in a total score in the range of 0-140 for each subscale. A higher score on both coping mechanisms indicate a greater tendency toward the respective type of coping ability.

2.1.3 Psychoacoustics

All participating subjects performed an Adaptive Categorical Loudness Scaling test. The tinnitus patients additionally performed a tinnitus test.

Psychoacoustic testing was performed using Tucker Davis Technologies equipment controlled by software developed in MatLab 2006b. The Tucker Davis Technologies setup consisted of a real-time processor (RP2), a headphones buffer (HB7) and two programmable attenuators (PA5). The stimuli were presented using TDH-49 headphones with MX-41/AR ear cushions. Subjects could respond by turning and subsequently pressing a bidirectional dial (Powermate USB Multimedia controller, Griffin Technologies).

Before psychoacoustic testing, all subjects first performed a training session. This procedure was developed to train the subjects in how to handle the response knob. Furthermore, the subject could become accustomed to the several types of questions and response scales that appear in the test. The subjects were asked to try how the scales on the screen respond to the movement of the response knob. Besides, the subjects were given control over the loudness and pitch of a stimulus to learn the meaning of the terms *loudness* and *pitch*.

All Subjects

Each subject performed the *Adaptive Categorical Loudness Scaling* (ACALOS) test. The purpose of the ACALOS test (Brand and Hohmann, 2002) is to estimate

loudness functions. A loudness function describes the relationship between sound intensity and loudness perception for the listener's entire dynamic range (i.e., from the threshold to the upper limit of comfort). Consequentially, one may expect that steeper slopes will be obtained in subjects with auditory hypersensitivity (i.e. hyperacusis) and in subjects with hearing loss (i.e. recruitment).

Measuring these loudness functions, white noise stimuli were presented twice for two seconds with a silence of one second in between. The response scale was permanently visible to the subject (see **Figure 2.1**). This response scale contained 11 response alternatives, of which seven were labeled. The labeled ones were 'inaudible', 'very soft', 'soft', 'medium', 'loud', 'very loud' and 'too loud', in accordance with 'onhoorbaar', 'zeer zacht', 'zacht', 'middel', 'luid', 'zeer luid' and 'te luid' in Dutch. The response alternatives corresponded to an internal loudness category ranging from zero up to and including 50, in steps of five. These loudness category numbers were used in the calculation of the final ACALOS function, but were not visible to the subject.

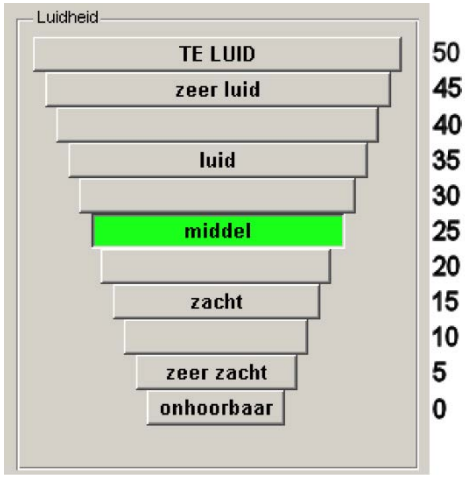


Figure 2.1. The ACALOS response scale with 11 response alternatives. The numbers on the right side indicate the loudness categories that were used for the calculation of the final ACALOS function. These numbers were not visible to the subjects.

During the presentation of a sound, the lighted selection bar was red. When the entire stimulus of five seconds in duration had ended, the color of the selection bar changed from red to green. Then, the subject could choose the matching answer by moving the selection bar over the respective response alternative and pressing

the dial. In contrast, the response alternative ‘too loud’ was always available and always shown in green when the selection bar is moved over it. Pressing the ‘too loud’ alternative stopped the stimulus immediately. Such a response was remembered as an absolute maximum and the program never exceeded a level that was once rated as ‘too loud’.

The procedure started with a level of 70 dB SPL. If this level was rated by the subject as ‘too loud’ or ‘inaudible’, the level changed in steps of 15 dB until the subject gave a rating between ‘very soft’ and ‘very loud’. Successively, both an ascending and a descending alternating sequence started. The software alternated between both sequences every other stimulus presentation. Concerning the ascending sequence, a step size of 10 dB was applied below 80 dB SPL and of 5 dB above this level. The ascending sequence ended if either the maximum allowed level (i.e. 110 dB SPL) was reached or the subject perceived the stimulus as ‘too loud’. Regarding the descending sequence, a step size of 15 dB was applied until the sound was rated as ‘inaudible’. In this situation, the descending sequence turned into an ascending one with a step size of 5 dB until the sound was rated as audible. The descending sequence ended if either the direction was converted into an ascending sequence or the minimum allowed level (−10 dB SPL) was reached.

Tinnitus Subjects

All tinnitus patients performed a *tinnitus test* based on computer-based tools as described by Roberts et al. (2006). The computerized test evaluates the quality of the tinnitus sensation and consists of seven steps completed in the order specified below. If the tinnitus was perceived on both ears, the respective subjects performed the test on each ear separately.

In the first step the subjects were asked on which ear the tinnitus is predominantly perceived (left/right/both ears). Second, the patient was instructed to set the level of a 1-kHz tone to a comfortable loudness level. The indicated loudness level was used in steps three and four. Third, subjects were asked to indicate whether they perceived the tinnitus as ‘tonal’, ‘hissing’ or ‘ringing’. An audio example was played consisting of respectively a pure tone, a narrow band noise (NBN10, ‘hissing’) or a very narrow band noise (NBN3, ‘ringing’). The NBN10 narrow band noise was produced by filtering Gaussian noise with a second-order Butterworth band pass filter set to a bandwidth of 10% of the center frequency (CF) at the −3-dB roll-off point. The NBN3 very narrow band noise was produced by filtering the Gaussian noise with two second-order Butterworth band pass filters in

series. The latter two filters were set to a bandwidth of 3% of the CF at the −3-dB roll-off point.

Next, the temporal properties of the tinnitus percept were considered. The subjects were asked to indicate whether their tinnitus was steady or pulsing. Audio examples of both choices were presented consisting of the sound type selected in the previous step. In the fifth step, the general tinnitus loudness was rated by selecting a position on a Borg CR100 scale (Borg and Borg, 2002) from −50 to 50 consisting of five labels: 'very soft' (−50), 'soft' (−20), 'moderate' (0), 'loud' (20) and 'very loud' (50), according to 'zeer zacht', 'zacht', 'middel', 'luid' and 'zeer luid' in Dutch.

The sixth step involved a tinnitus loudness matching procedure. Nine sound stimuli with CFs ranging from 0.5 to 10 kHz were presented: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 and 10 kHz. The loudness matching procedure was randomly repeated three times for each stimulus frequency. The bandwidth of the stimuli was determined by the answer given by the subject in step 3. The subjects were instructed to adjust the loudness of each stimulus in steps of 1 dB to match the loudness of their tinnitus. Pressing the dial submitted the response and proceeded to the next stimulus. An average matching loudness was calculated for each stimulus.

In the last step, the stimuli were presented in a random order at the respective average matched loudness. Again, each stimulus frequency was presented three times. The subjects were asked to give a likeness rating to each stimulus by responding the question "Is this sound part of your tinnitus?" Yet again, a Borg CR100 scale was used for the similarity rating with five labels: 'no', 'a little bit', 'moderate', 'clearly', 'identical', in accordance with 'niet', 'klein beetje', 'matig', 'duidelijk' and 'identiek' in Dutch. As such, a profile of the tinnitus likeness was generated by averaging the three likeness ratings for each stimulus frequency. This likeness profile is referred to as a tinnitus spectrum.

2.2 Results

Significant differences between both groups concerning hearing thresholds, questionnaires and ACALOS measures were assessed by two-tailed two-sample *t*-tests and Fisher's exact test. Statistical relationships between the various questionnaires were determined by means of Pearson correlations.

Table 2.1. Subjects' characteristics. Hearing loss was measured as the pure-tone average (PTA) hearing threshold at the octave frequencies 1.0, 2.0 and 4.0 kHz. The mean values with standard deviation, and the *p*-values are listed. Group differences concerning 'Age' and 'Hearing Loss' were calculated by means of a two-sample *t*-test. Group differences concerning 'Gender' and 'Handedness' were tested by means of Fisher's exact test. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

	HI+T (n = 34)	HI (n = 19)	*p*-values
Age			
years	57 ± 10	62 ± 12	0.07
range	31 → 75	44 → 84	
Gender			
male	21	16	0.12
female	13	3	
Handedness			
right	28	16	
left	2	1	1.00
ambidextrous	4	2	
Hearing Loss (dB HL)			
right ear	41 ± 8	44 ± 11	0.20
left ear	42 ± 10	45 ± 8	0.17

2.2.1 Population characteristics

For the HI+T group and the HI group, 34, respectively 19 subjects met the inclusion criterion with regard to hearing loss and were enrolled in the study. Details of the participant characteristics are listed in **Table 2.1**. No significant difference in population characteristics existed. Age was significantly correlated with hearing loss (left ear: $R = 0.37$, $p = 0.007$; right ear: $R = 0.34$, $p = 0.012$).

The mean audiogram per patient group is shown in **Figure 2.2**. Statistical analysis of the hearing threshold at each octave frequency respectively did not show significant differences between both groups.

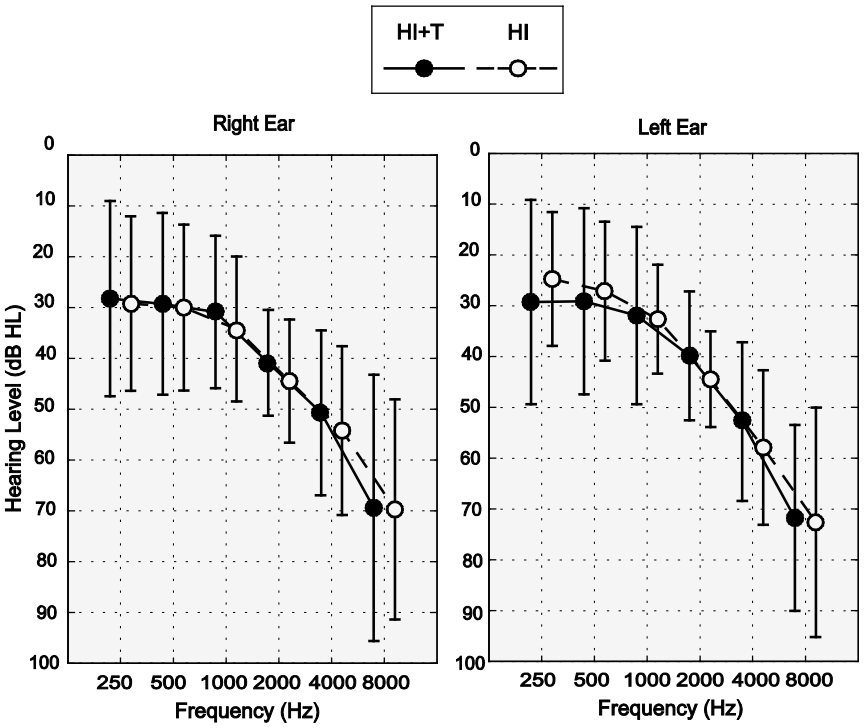


Figure 2.2. Mean audiograms for the HI+T group and HI group. The error bars indicate the group standard deviations around the mean. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

2.2.2 Perceptual tinnitus characteristics

Approximately 73% (25 out of 34) of the tinnitus patients perceived tinnitus bilaterally. About 21% (7 subjects) reported perceiving their tinnitus in the left ear or left side of the head, and only 6% (2 subjects) in the right ear or right side of the head. The tinnitus sound was most often described as hissing (61% of the total of 59 ears), followed by tonal (32% of the ears) and ringing (7% of the ears). All patients specified that their tinnitus was non-pulsatile in nature. The subjectively perceived tinnitus loudness before and after scanning is shown in **Figure 2.3**. On a scale from 0-10, the tinnitus loudness was rated as 5.2±2.0 (mean±SD) before scanning and as 5.5±1.9 after scanning. Thus, the tinnitus loudness was slightly increased after scanning the subjects. Both measurements were highly correlated ($R = 0.84$, $p << 0.001$). Compared to before scanning, the rating after scanning included loudness increases (29%; 10 subjects) as well as decreases (15%; 5 subjects). However, 56% (19 out of the 34) of the tinnitus subjects did not report any loudness change.

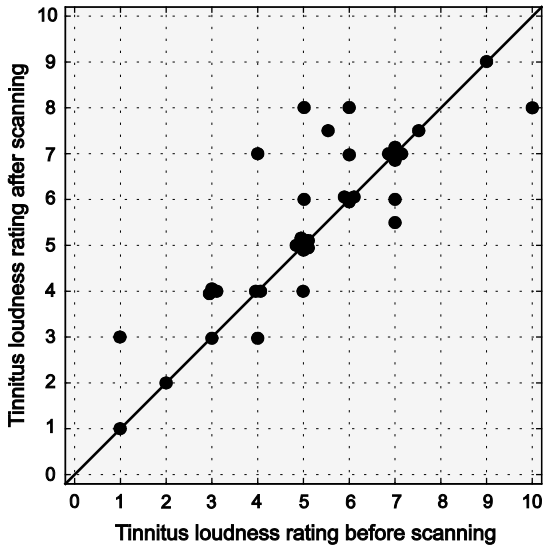


Figure 2.3. Tinnitus loudness ratings before and after scanning. The ratings were measured by means of a numeric rating scale (NRS) from 0 (inaudible) to 10 (tinnitus sounds as loud as imaginable).

2.2.3 Questionnaire outcomes

The outcomes of the questionnaires are listed in **Table 2.2** and visualized in **Figure 2.4**.

On average, the HI+T group had higher scores on both subscales of the HADS than the HI group. These group differences were statistically significant (see **Table 2.2**). In the HI group, the HADS score exceeded the clinical threshold (i.e. >8) for anxiety and depression in 11% of the subjects (2 out of 19) and 5% of the subjects (1 out of 19), respectively. In the HI+T group, the HADS score exceeded the clinical threshold (i.e. >8) for anxiety and depression in 21% of the subjects (7 out of 34) and 26% of the subjects (9 out of 34), respectively.

Auditory hypersensitivity, that is hyperacusis, was measured by means of the HQ. The HQ was filled out by all but two subjects. These two subjects belonged to the HI+T group. On the HQ, the tinnitus subjects had on average higher scores than the hearing-impaired controls. This group difference was significant, with the tinnitus patients suffering more from auditory hypersensitivity than those without tinnitus (see **Table 2.2**). The average HQ score of both groups was below the clinical cut-off score of 28. In the HI and HI+T group, 5% of the subjects (1 out of 19) and 18% of the subjects (6 out of 34), respectively, exceeded this threshold.

The THI was significantly correlated with the TRQ ($R = 0.87$; $p < 0.001$) and the $TCSQ_{mal}$ ($R = 0.82$; $p < 0.001$), but not with the $TCSQ_{effect}$ ($R = 0.26$; $p > 0.05$). Similarly, the TRQ was significantly correlated with the $TCSQ_{mal}$ ($R = 0.90$; $p < 0.001$), but not with the $TCSQ_{effect}$ ($R = 0.33$; $p > 0.05$). Both subscales of the TCSQ did not show a significant correlation ($R = 0.33$; $p > 0.05$). These results show that all three questionnaires measure the reaction to tinnitus in a similar way.

Table 2.2. Questionnaire outcomes. The mean values with standard deviation and the *p*-values are listed. Group differences concerning 'HADS-A', 'HADS-D' and 'HQ Score' were calculated by means of a two-sample *t*-test. HADS-A: Hospital Anxiety and Depression Scale–Anxiety; HADS-D: Hospital Anxiety and Depression Scale–Depression; HQ: Hyperacusis Questionnaire; THI: Tinnitus Handicap Inventory; TRQ: Tinnitus Reaction Questionnaire; $TCSQ_{effect}$: Tinnitus Coping Style Questionnaire effective coping; $TCSQ_{mal}$: Tinnitus Coping Style Questionnaire maladaptive coping; HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

	HI+T (n = 34)	HI (n = 19)	*p*-values
HADS-A (0 → 21)			
score	5 ± 4	3 ± 3	0.038
range	0 → 16	0 → 10	
HADS-D (0 → 21)			
score	5 ± 4	1 ± 3	0.006
range	0 → 13	0 → 9	
HQ Score (0 → 42)			
score	20 ± 7	15 ± 8	0.024
range	5 → 33	2 → 30	
THI Score (0 → 100)			
score	31 ± 22	–	–
range	4 → 72		
TRQ Score (0 → 104)			
score	27 ± 22	–	–
range	0 → 75		
$TCSQ_{mal}$ Score (0 → 120)			
score	25 ± 16	–	–
range	3 → 67		
$TCSQ_{effect}$ Score (0 → 120)			
score	56 ± 18	–	–
range	1 → 80		

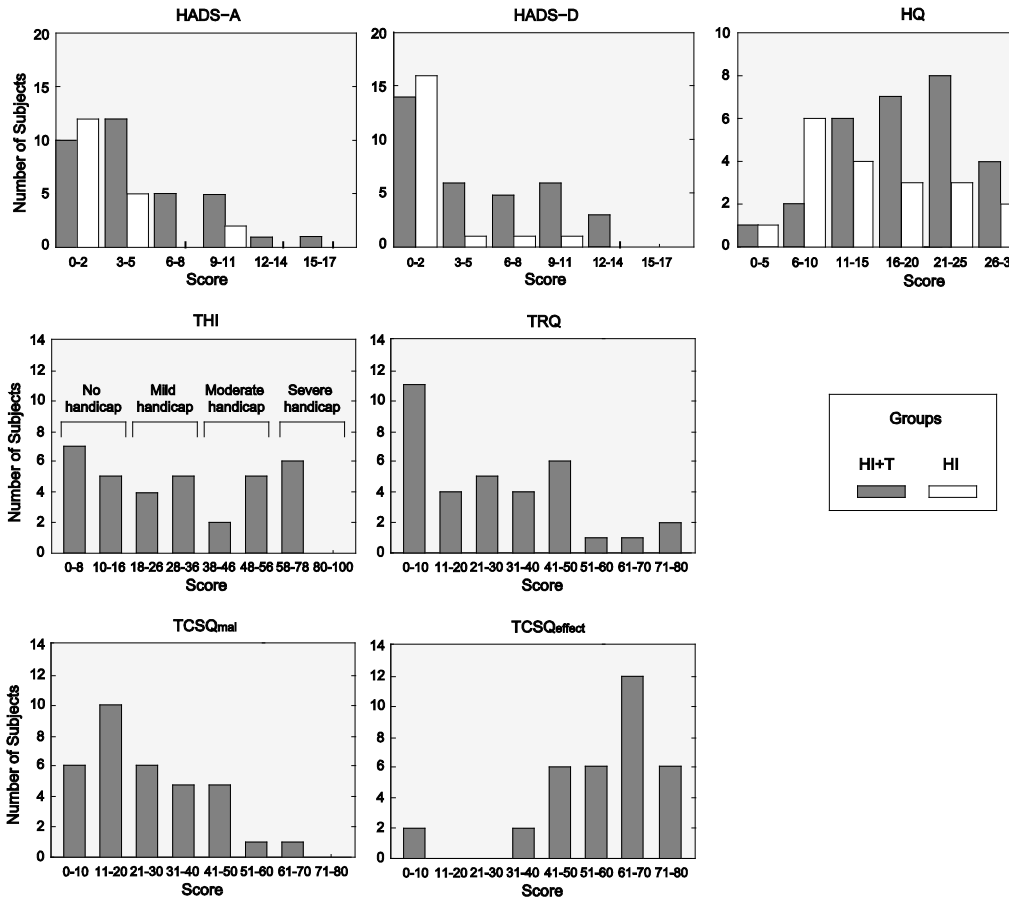


Figure 2.4. Distribution of the questionnaire outcomes. The number of subjects as function of the questionnaire scores is shown. HADS-A: Hospital Anxiety and Depression Scale–Anxiety; HADS-D: Hospital Anxiety and Depression Scale–Depression; HQ: Hyperacusis Questionnaire; THI: Tinnitus Handicap Inventory; TRQ: Tinnitus Reaction Questionnaire; $TCSQ_{effect}$: Tinnitus Coping Style Questionnaire effective coping; $TCSQ_{mal}$: Tinnitus Coping Style Questionnaire maladaptive coping; HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

2.2.4 Psychoacoustics

The results of the ACALOS loudness scaling procedure are shown in **Figure 2.5**. Louder stimulus levels were clearly attributed to higher stimulus categories. The ACALOS curves do not show clear differences between both hearing-impaired groups. The only significant group difference was found for loudness category 3 when the stimuli were presented at the right ear. Compared to the HI group, the HI+T group placed higher stimulus levels in the intermediate loudness category between 'very soft' and 'soft' (p = 0.037, uncorrected for multiple comparisons).

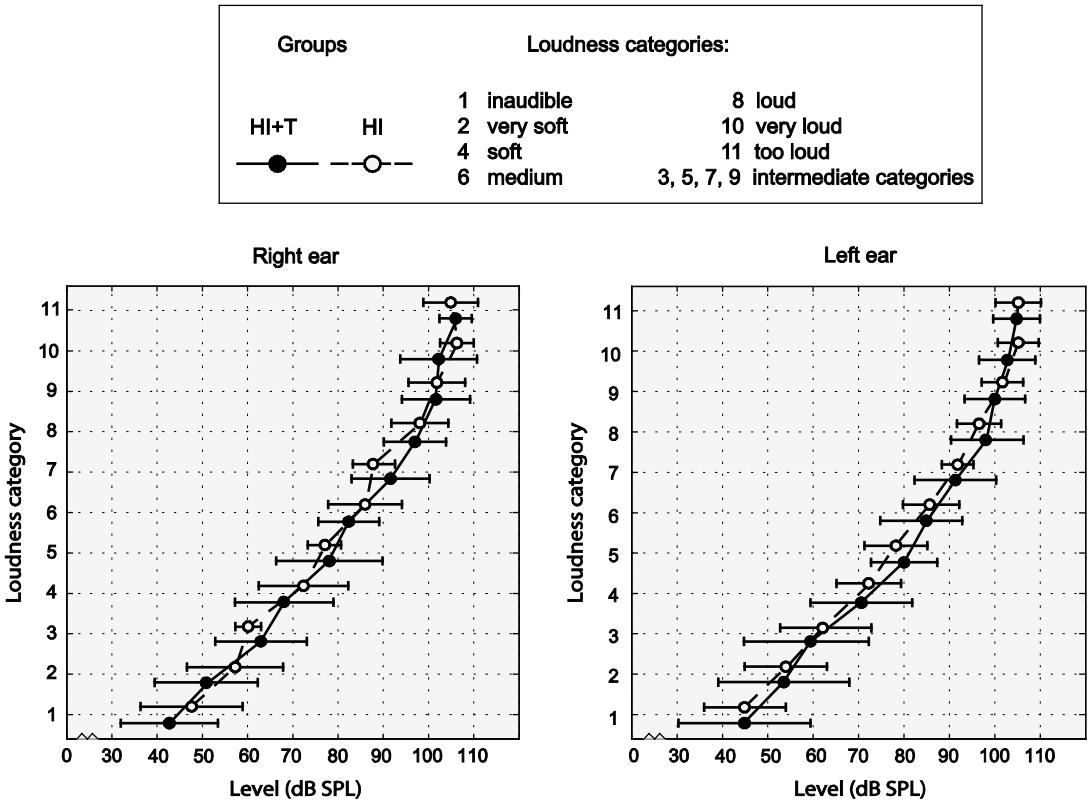


Figure 2.5. Loudness functions as measured by the ACALOS test for the HI+T group and HI group. The error bars indicate the group standard deviations around the mean. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

An overview of the response percentages per loudness category, group and ear is listed in **Table 2.3**. This overview obviously shows a response bias to the labeled loudness categories. Furthermore, about half of the tinnitus patients rated a stimulus level as 'too loud'; whereas the percentages for that loudness category with regard to the HI group were lower. This indicates that more tinnitus patients perceived a stimulus level as 'too loud'.

Table 2.3. Answer percentages per ACALOS loudness category, group and ear. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired.

Stimulus categories		HI+T (n = 34)		HI (n = 19)	
		Right ear [%]	Left ear [%]	Right ear [%]	Left ear [%]
1	Inaudible	100	100	100	100
2	Very soft	100	100	100	100
3	Intermediate	38	35	12	21
4	Soft	100	100	100	95
5	Intermediate	47	44	37	58
6	Medium	100	100	100	100
7	Intermediate	59	65	42	47
8	Loud	100	97	95	100
9	Intermediate	47	38	32	47
10	Very loud	76	82	89	89
11	Too loud	53	50	26	47

Figure 2.6 shows the results of the tinnitus test. Both the outcomes of the loudness matching and the likeness indication are displayed. Both measures showed a considerable individual variability. For the loudness matching curves, the matched loudness generally increased with increasing CF ranging from 2.2 dB HL at CF 0.5 kHz, to 90 dB HL at CFs 5.0, 6.0, 8.0 and 10 kHz. Because all our tinnitus patients had high-frequency hearing loss, loudness categories chosen by the subjects are likely to be higher for sounds of high CF. However, despite the ascending audiograms, the loudness matching curves are comparatively flat.

For both ears, mean likeness ratings increased for higher CF and reached an averaged maximum at CF 5.0 kHz (right ear) and at CF 6.0 kHz (left ear) before diminishing at higher CFs. Overall, the tinnitus likeness ratings spanned the region of measured hearing loss (threshold > 30 dB HL).

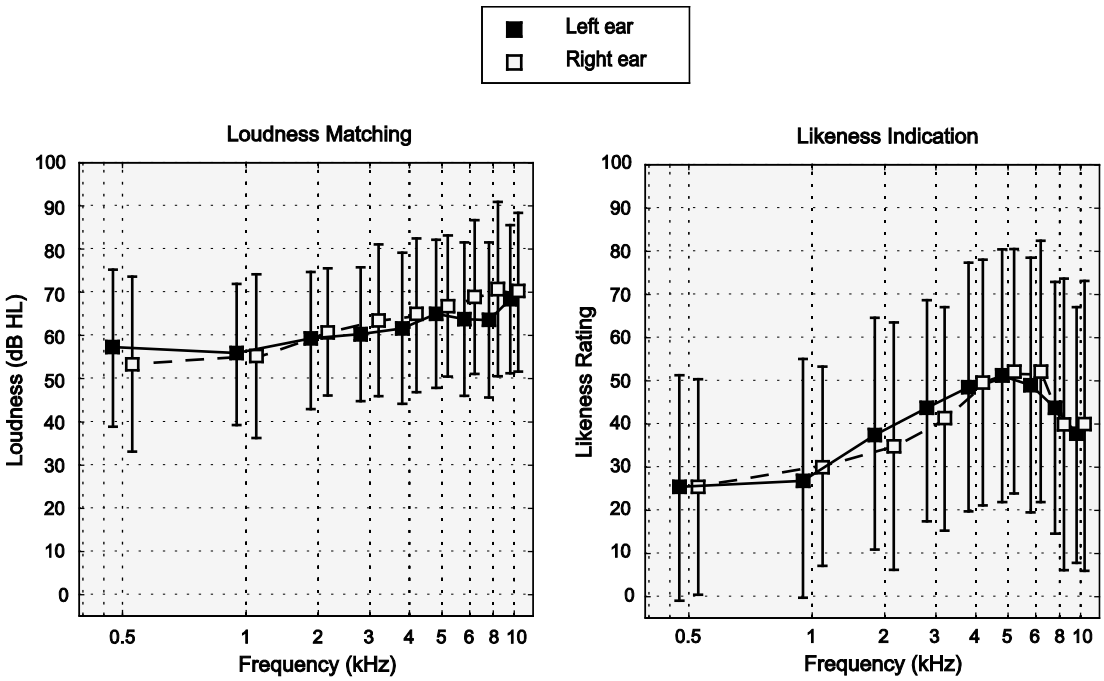


Figure 2.6. The loudness matching and likeness curves as measured by the tinnitus test. Mean curves for the left and right ear are presented using a logarithmic scale for the x-axis. The error bars indicate the group standard deviations around the mean.

2.3 Conclusions

All subjects participated in an extensive test battery comprising of questionnaires and psychoacoustic tests. Subjects were included in order to match the groups with respect to age and hearing loss. As follows from statistical analyses, there were no significant differences between the groups relating to age and hearing loss. Moreover, no significant differences relating to gender and handedness were found.

The two subject groups did, however, differ regarding sound tolerance and anxiety and depression. These differences are presumably related to the presence of tinnitus, which is known to be associated with reduced sound tolerance (Baguley, 2003) and anxiety and depression (Zöger et al., 2001; Dobie, 2003). All tinnitus subjects perceived a subjective non-pulsatile tinnitus, which was predominantly high-frequent. The tinnitus questionnaires indicated mild tinnitus in the majority of the subjects.

Interestingly, the reduced sound tolerance as found in the tinnitus group was not confirmed by the slopes of the ACALOS test. However, the tinnitus patients perceived more often a stimulus level as 'too loud'. Apparently, the ACALOS test does not reflect the reduced sound tolerance as indicated by the HQ in terms of steeper slopes, but probably in terms of perceiving stimulus levels as 'too loud'.

Chapter 3

Gray Matter in the Human Brain: Differences Associated with Tinnitus and Hearing Loss

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ABSTRACT

Tinnitus, usually associated with hearing loss, is characterized by the perception of sound without an external sound source. The pathophysiology of tinnitus is poorly understood. In the present study, voxel-based morphometry (VBM) was employed to identify gray matter differences related to hearing loss and tinnitus. VBM was applied to magnetic resonance images of normal-hearing control subjects (n=24), hearing-impaired subjects without tinnitus (n=16, HI group) and hearing-impaired subjects with tinnitus (n=31, HI+T group). This design allowed us to disentangle the gray matter (GM) differences related to hearing loss and tinnitus, respectively. Voxel-based VBM analyses revealed that both HI and HI+T groups, relative to the controls, had GM increases in the superior and middle temporal gyri, and decreases in the superior frontal gyrus, occipital lobe and hypothalamus. We did not find significant GM differences between both patient groups. Subsequent region-of-interest (ROI) analyses of all Brodmann Areas, the cerebellum and the subcortical auditory nuclei showed a GM increase in the left primary auditory cortex of the tinnitus patients compared to the HI and control groups. Moreover, GM decreases were observed in frontal areas and mainly GM increases in limbic areas, both of which occurred for hearing loss irrespective of tinnitus, relative to the controls. These results suggest a specific role of the left primary auditory cortex and the additional involvement of various non-auditory brain structures in tinnitus. Understanding the causal relation between these GM changes and tinnitus will be an important next step in understanding tinnitus mechanisms.

3.1 Introduction

Tinnitus, the perception of sound without an external source, is a common disorder. Prevalence estimates generally range from 7 to 20% (Hoffman and Reed, 2004). Tinnitus may be mild but may also have a devastating impact on the ability to function in daily life, leading many patients to seek medical attention (Lockwood et al., 2002). A variety of additional symptoms is often reported, including stress, anxiety, depression, insomnia and irritability (Møller, 2000; Hébert and Lupien, 2007; Langguth et al., 2011). Approximately 40% of the patients with a primary complaint of tinnitus suffer from hyperacusis, an intolerance to loud sounds, as well (Baguley, 2003).

The underlying pathophysiology of tinnitus is still poorly understood. Aging or loud-noise exposure, both of which may lead to hearing loss, are often associated with tinnitus. Since the associated hearing loss usually has a peripheral origin, the generator of tinnitus initially was thought to lie in the inner ear. However, since dissection of the vestibulocochlear nerve does not eliminate the tinnitus in the majority of subjects (House and Brackmann, 1981; Berliner et al., 1992), an important role in the generation of tinnitus is currently attributed to mechanisms in the central auditory system. Further support for tinnitus generation in the central auditory system is provided by a number of studies that employed positron-emission tomography (Arnold et al., 1996; Giraud et al., 1999; Wang et al., 2001; Langguth et al., 2006) and functional magnetic resonance imaging (Melcher et al., 2000; Lanting et al.; 2008; Melcher et al., 2009), demonstrating changes in the inferior colliculus, thalamus and auditory cortex (for reviews see Lanting et al., 2009 and Adjamian et al., 2009). Imaging studies further indicate that non-auditory brain areas may play a role in tinnitus. The limbic system in particular has been shown to exhibit abnormal activity in tinnitus patients (Andersson et al., 2000).

If tinnitus is based on functional properties of the brain, there are presumably neuroanatomical correlates of tinnitus as well. A relatively new method to study the neuroanatomy of the human brain is voxel-based morphometry (VBM). VBM was introduced to demonstrate differences in the amount of gray and white matter of brain regions among subject populations on the basis of high-resolution structural magnetic resonance images (Ashburner and Friston, 2000; Good et al., 2001). The regional *absolute* amount of gray matter is commonly referred to as gray matter *volume*, whereas the regional *relative* amount of gray matter (as opposed to other tissue types) is referred to as gray matter *concentration* (Ashburner and Friston, 2000). May and Gaser (2006) argued that

simple changes in cell size, growth or atrophy of neurons or glia cells as well as changes in the intra-cortical axonal architecture may be potential correlates of observed morphometric differences.

Up till now, five studies have investigated alterations in gray matter in tinnitus patients. Mühlau et al. (2006), Landgrebe et al. (2009), Schneider et al. (2009), Husain et al. (2011) and Leaver et al. (2011) observed gray matter changes in auditory as well as non-auditory brain areas. An overview of these studies is listed in **Table 3.1**. Two notable differences between the studies may be indicated. First, the characteristics of the participating subject groups vary across studies, in particular with respect to tinnitus severity, the occurrence of co-morbid hearing loss, and group size. Second, the specific structures in which alterations were found are inconsistent across studies, but include temporal, frontal and limbic regions. Differences in the auditory system were found by Landgrebe et al. (2009), Schneider et al. (2009) and Husain et al. (2011). Both Mühlau et al. (2006) and Leaver et al. (2011) observed a gray matter decrease in the subcallosal area for tinnitus subjects compared to controls. Differences in the thalamus, the hippocampus, and the frontal areas were observed by Mühlau et al. (2006), Landgrebe et al. (2009) and Husain et al. (2011) respectively.

The inconsistencies between the previous studies prompted us to set up a new VBM study to gain more insight in the neuroanatomical differences related to tinnitus and hearing loss. In our study, three relatively large subject groups were included: a hearing-impaired tinnitus group (n = 31), a hearing-impaired non-tinnitus group (n = 16), and a normal-hearing control group (n = 24). Both hearing-impaired groups were carefully matched with respect to hearing loss. This design allowed us to identify the effects that are specific to hearing loss and tinnitus respectively. We primarily focused on the auditory system, but also considered the rest of the brain. Based on previous studies, we expected to find differences in auditory, limbic, paralimbic and frontal regions.

Table 3.1. Overview of previous VBM studies. TIN: tinnitus, not hearing-impaired; HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired; C: controls; GHQ: Goebel-Hiller Questionnaire (Goebel and Hiller, 1994); TCQ: Tinnitus Characteristics Questionnaire (see Leaver et al., 2011); MASK: a mask of the auditory or limbic system was used (see Mühlau et al., 2006; Landgrebe et al., 2009; Husain et al., 2011); post. Thal.: posterior thalamus; IC: inferior colliculus; Hipp.: hippocampus; BCC: bilateral cingular cortex; AntC: anterior cingular cortex; BMFG: bilateral medial frontal gyrus; BSFG: bilateral superior frontal gyrus; BSTG: bilateral superior temporal gyrus; PTA: pure-tone average; SFG: superior frontal gyrus; vmPFC: ventero-medial prefrontal cortex; mHG: medial Heschl's gyrus.

Subjects' characteristics						Analysis			Gray matter	
Subject groups	**# subjects**	**Hearing Loss**	**Mean age**	**Gender**	**Tinnitus severity**	**Analysis type**	**Volume correction**	**Threshold (voxel-level)**	**Modulated Images**	**Unmodulated Images**
Mühlau et al. (2006)										
TIN	28	No	40	15♀ 13♂	GHQ: Mean score: 25	Voxel-by-voxel	No	$p < 0.05$ corrected	TIN<C: Subcallosal area	-
C	28	No	39	15♀ 13♂	-	Mask	Yes	$p < 0.05$ corrected	-	TIN>C: Right post.Thal. incl. MGB
								$p < 0.05$ uncorrected	-	TIN>C: Left post. Thal.
Landgrebe et al. (2009)										
TIN	28	No	32	13♀ 15♂	GHQ: Mean score: 32.9	Voxel-by-voxel	No	$p < 0.05$ FDR-corrected	-	-
C	28	No	31	13♀ 15♂	-	Mask	Yes	$p < 0.05$ corrected	-	TIN<C: Right IC; Left Hipp.
								$p < 0.05$ uncorrected	-	TIN<C: Right Hipp.; BCC
Husain et al. (2011)										
HI+T	8	Moderate-to-moderately severe from 2 to 8kHz	56	0♀ 8♂	THI Mean score: 17.25	Voxel-by-voxel	Yes	$p < 0.001$ uncorrected	HI<C: Right AntC; BMFG	-
HI	7	Moderate-to-moderately severe from 2 to 8 kHz	51	0♀ 7♂	-				HI<HI+T: BSFG; BMFG	
C	11	No	48	0♀ 11♂	-	Mask	Yes	$p < 0.005$ uncorrected	HI<C: BSTG	-
									HI<HI+T: BSTG	
								$p < 0.001$ uncorrected (PTA as a regressor)	HI<C: Left SFG & BMFG	-
									HI<HI+T: Central SFG & BMFG	
Leaver et al. (2011)										
TIN	11	28.11 dB SPL	44	6♀ 5♂	TCQ: Mean score: 2.45	Voxel-by-voxel	Yes	$p < 0.0001$ uncorrected	TIN<C: vmPFC (subcallosal region)	TIN<C: vmPFC (subcallosal region)
C	11	10.74 dB SPL	23	7♀ 4♂						
Schneider et al. (2009)										
TIN	61	HL≤ 15 dB HL: 16	44	20♀ 41♂	GHQ: Mean score: 23.2	Structural Segmentation Method			TIN<C: mHG	-
C	45	HL≤ 15 dB HL: 29	39	23♀ 22♂	-					

3.2 Materials and methods

3.2.1 Subjects

This study included data collected from two groups of patients and a control group. The patients were recruited at the University Medical Center Groningen (UMCG) and via hearing aid dispensers in Groningen, The Netherlands. The first group comprised 16 hearing-impaired subjects (HI group). The second group consisted of 31 subjects with a hearing impairment and tinnitus (HI+T group). Pure-tone audiometry was performed with a clinical audiometer using six different octave frequencies (0.25, 0.5, 1, 2, 4 and 8 kHz). Hearing-impaired subjects were included if the pure-tone average (PTA) hearing threshold at the octave frequencies 1, 2 and 4 kHz satisfied $30 \leq PTA \leq 60$ dB HL in both ears. Additionally, compatible MRI data that were available at our institute were included. These comprised 24 healthy controls with normal hearing; no further psychoacoustic outcomes were obtained for this group.

Further details of the subjects were assessed by three questionnaires that examine tinnitus handicap, hyperacusis, and handedness, respectively (see **Table 3.2**). In addition, all tinnitus subjects were asked about the duration, lateralization and dynamic nature of their tinnitus.

Tinnitus handicap was assessed by a Dutch translation of the Tinnitus Handicap Inventory (THI), a self-reported tinnitus handicap questionnaire (Newman et al., 1996). The 25-item THI consists of questions examining the functional, emotional and catastrophic reactions to tinnitus. The total THI score ranges from 0 to 100. Higher total scores represent a higher impact of the tinnitus on everyday life. In order to assess the presence of hyperacusis, a translated version of the Hyperacusis Questionnaire (HQ; Khalfa et al., 2002) was administered. The 14-item HQ consists of questions examining the attentional, social and emotional dimensions of hyperacusis. The total HQ score ranges from 0 to 42. Higher total scores represent a stronger auditory hypersensitivity. To assess handedness, a translated version of the Edinburgh Inventory (Oldfield, 1971) was completed by all subjects. None of the subjects had any major medical, neurological or psychiatric history.

This study was approved by the medical ethics committee of the University Medical Center Groningen. All subjects gave written informed consent in accordance with Dutch legislation.

3.2.2 Data acquisition

A 3-T Philips Intera MR-scanner (Philips Medical Systems, Best, The Netherlands) with an eight-channel phased-array (SENSE) headcoil at the Neuroimaging Center of the University Medical Center Groningen was used to collect structural brain images. For each subject, a 3-dimensional high-resolution *T*1-weighted fast-field echo scan was acquired (170 slices; acquisition duration 251 s; repetition time (TR) 9 ms; echo time (TE) 3.50 ms; flip-angle 8°; matrix 256 x 256; voxel size 1 x 1 x 1 mm^3).

3.2.3 Data processing

Data processing was performed using the VBM5 toolbox within the SPM5 software package (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK) running under MatLab 7.1. The VBM5 toolbox provides a framework integrating spatial normalization, segmentation and MRI inhomogeneity bias correction of the data (Ashburner and Friston, 2005). First, spatial normalization onto the Montreal Neurological Institute (MNI) stereotactic space was applied to each brain, by registering all images to the ICBM 452 *T*1 template image (Ashburner and Friston, 2000). Thereafter, MRI inhomogeneity bias correction and segmentation of the images were simultaneously performed. The spatially normalized images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). During the tissue classification, the intensity distributions in the acquired individual MRI images are combined with the standard prior probabilities for voxels to belong to either GM, WM or CSF compartments. From this, posterior probability maps are derived for voxels to belong to each of the aforementioned three tissue types. Because of our interest in gray matter, only the GM images were analyzed further.

During spatial normalization, volumetric distortions (due to stretching and shrinking) occur when matching an individual image to the template. This may lead to misrepresentations regarding the total amount of GM in an image. In order to remove this confound, modulation was applied. This involves scaling the images by the amount of local volumetric contraction or expansion in order to preserve the total designated amount of GM in the normalized images (Ashburner and Friston, 2000).

In the next step, the unmodulated as well as the modulated images were smoothed with an 8-mm isotropic Gaussian kernel. The whole procedure yielded

one smoothed unmodulated image and one smoothed modulated image per subject. Analysis of unmodulated data tests for regional differences in the posterior probability of a particular tissue as opposed to other tissue types, henceforth referred to as gray matter amount obtained from unmodulated images (GM-U), corresponding to the term 'concentration'. In contrast, the modulated data tests for regional differences in the volumetrically integrated amount of a particular tissue, henceforth referred to as gray matter amount obtained from modulated images (GM-M), corresponding to the term 'volume' (Ashburner and Friston, 2000; Mechelli et al., 2005). Whereas GM-U is restricted to a range of 0 to 1 in each voxel by definition, GM-M may exceed 1 if the subject's anatomy was locally compressed during the spatial normalization stage such that one normalized voxel corresponds with more than one voxel in the original acquired brain.

In order to avoid possible edge effects around the border between white and gray matter and to include only voxels with sufficient GM, all voxels with a GM value less than 0.2 were excluded. To correct for global brain differences between the groups, the GM-M (and GM-U) images were scaled by dividing the GM value of each voxel by the total GM of that image.

3.2.4 Statistical analysis

Following the preprocessing, the final step in VBM is to perform statistical analyses. Whole-brain voxel-by-voxel comparisons and a region-of-interest (ROI) analysis were performed. For each analysis, both modulated and unmodulated data were considered. Non-sphericity correction was applied in order to correct for heterogeneity of variance among the groups.

Whole-brain voxel-by-voxel comparisons

The GM images were analyzed using a general linear model in order to compare local GM characteristics of the three subject groups. To determine the main effect of group on gray matter, a one-way analysis of covariance (ANCOVA) regression model with GM-M or GM-U as the response variable, group membership as the explanatory variable of interest and age as additional explanatory variable of no interest was evaluated. A confidence threshold of $p < 0.05$ family-wise error (FWE) corrected for multiple comparisons was applied. Next, two-sample *t*-tests were performed masked by the ANCOVA main effects results in order to evaluate GM differences between the three subject groups. A confidence threshold of $p < 0.05$

FWE corrected was applied. Additionally, an extent threshold of 50 adjacent voxels was used.

ROI analyses

In the ROI analysis, GM-M and GM-U differences between the groups were examined for various subdivisions of the brain. We performed ROI analyses on 78 Brodmann Areas (BAs), as defined in both hemispheres separately according to the WFU_pickatlas (Maldjian et al., 2003). In addition, ROIs of eight subcortical auditory nuclei were included. These consisted of the left and right cochlear nuclei (sphere radius, 5 mm; MNI coordinates, ±10, -38, -45), the left and right superior olivary complex (sphere radius, 5 mm; MNI coordinates, ±13, -35, -41), the left and right inferior colliculus (sphere radius, 5 mm; MNI coordinates, ±6, -33, -11) and the left and right medial geniculate nucleus (sphere radius, 8 mm; MNI coordinates, ±17, -24, -2). These definitions conform to Mühlau et al. (2006), Landgrebe et al. (2009) and Husain et al. (2011). Additionally, two ROIs were included comprising the left and the right cerebellum, respectively. Both ROIs were defined according to the WFU_pickatlas.

For each ROI, the average GM-M and GM-U values were calculated for each subject. To determine the main effect of group on GM in the ROIs, ANCOVA regression models with GM-M or GM-U as the response variable, group membership as the explanatory variable of interest and age as additional explanatory variable of no interest were evaluated. A confidence threshold of $p < 0.05$ was applied, corrected for false discovery rate (FDR; Benjamini et al. (2001)). After the estimated age-related effects were subtracted, two-sample *t*-tests were performed on the residuals, masked by the ANCOVA main effects results, to evaluate differences between all pairs of groups. A confidence threshold of $p < 0.05$ uncorrected was applied.

3.3 Results

3.3.1 Subject characteristics

The mean audiogram per patient group is shown in **Figure 3.1**. Although the HI group had on average slightly worse thresholds, statistical analysis of the hearing threshold at the octave frequencies 1, 2 and 4 kHz did not show significant differences between both groups.

Details of the participants' characteristics are listed in **Table 3.2**. A significant difference in age existed between the three groups ($F = 3.51$, $p = 0.036$). Post-hoc tests showed a significant difference in age between the HI+T and HI groups ($p = 0.025$), and between the HI and control groups ($p = 0.046$), where in both cases the HI group was significantly older. Age was significantly correlated with hearing loss ($R = 0.30$, $p = 0.044$).

All but two subjects from the HI+T group filled out the HQ. A significant difference between both groups ($p = 0.026$) was found, with the HI+T group scoring higher relative to the HI group. In addition, a correlation between the THI and HQ scores was found ($R = 0.59$, $p = 8.1\times10^{-4}$). Out of all 31 tinnitus subjects, 22 subjects perceived tinnitus in both ears, seven subjects perceived tinnitus only in the left ear and two subjects perceived tinnitus only in the right ear. All tinnitus subjects had chronic continuous tinnitus with a duration ranging from one up to 29 years.

3.3.2 Whole-brain voxel-by-voxel comparisons

A whole-brain ANCOVA analysis was conducted. Next, two-sample *t*-tests were performed masked by the ANCOVA main effects result. For the whole-brain analysis, a confidence threshold of $p < 0.05$ FWE corrected for multiple comparisons was applied. The results are listed in **Table 3.3** and shown in **Figure 3.2**. For both the GM-M and GM-U measures, no significant differences were found between the HI+T and HI group. In contrast, both the GM-M and GM-U comparisons showed similar significant differences between the two hearing-impaired groups and the control group. GM increases were found in the superior temporal gyrus for both hearing-impaired groups compared to the control group; decreases were found in the occipital lobe and hypothalamus.

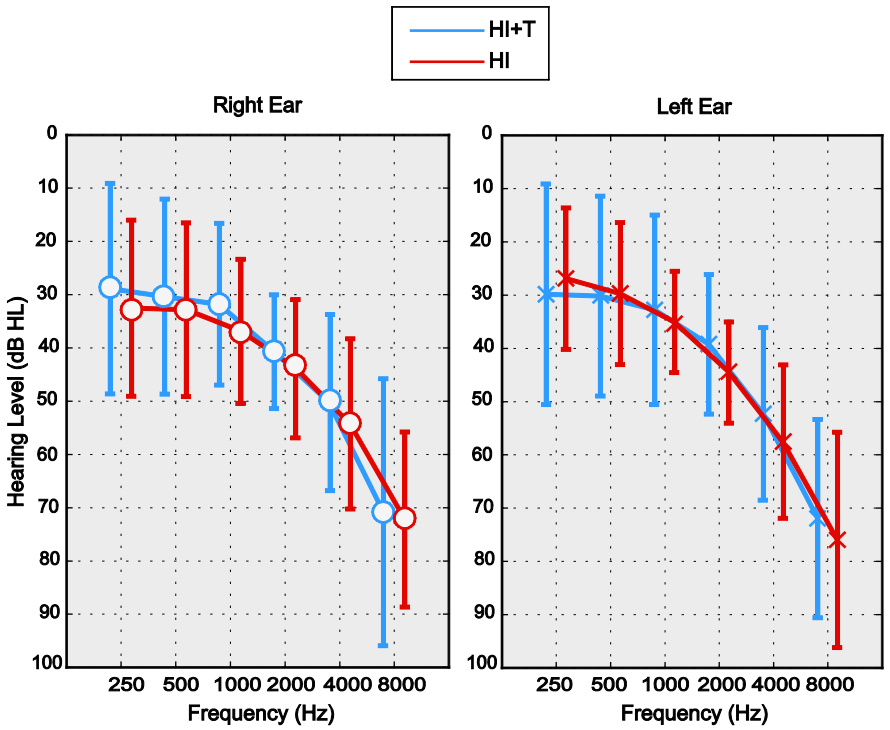

Figure 3.1. Mean audiograms for the HI+T group (blue line) and HI group (red line). The error bars indicate the group standard deviations around the mean. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects.

Table 3.2. Subjects' characteristics; mean value with standard deviation. For all hearing-impaired subjects, the hearing loss was measured as the pure-tone average (PTA) hearing threshold at the octave frequencies 1, 2 and 4 kHz. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired; HQ: Hyperacusis Questionnaire, THI: Tinnitus Handicap Inventory.

	HI+T (n = 31)	HI (n = 16)	Controls (n = 24)
Age			
years	56 ± 9	63 ± 10	58 ± 6
range	31 → 75	44 → 84	50 → 69
Gender			
male	20	13	16
female	11	3	8
Handedness			
right	27	13	24
left	1	1	0
ambidextrous	3	2	0
Hearing Loss (dB HL)			
right ear	41 ± 8	45 ± 11	-
left ear	41 ± 10	46 ± 10	
HQ Score (0 → 42)			
score	20 ± 7	14 ± 7	-
range	5 → 32	2 → 27	
THI Score (0 → 100)			
score	29 ± 20	-	-
range	4 → 72		

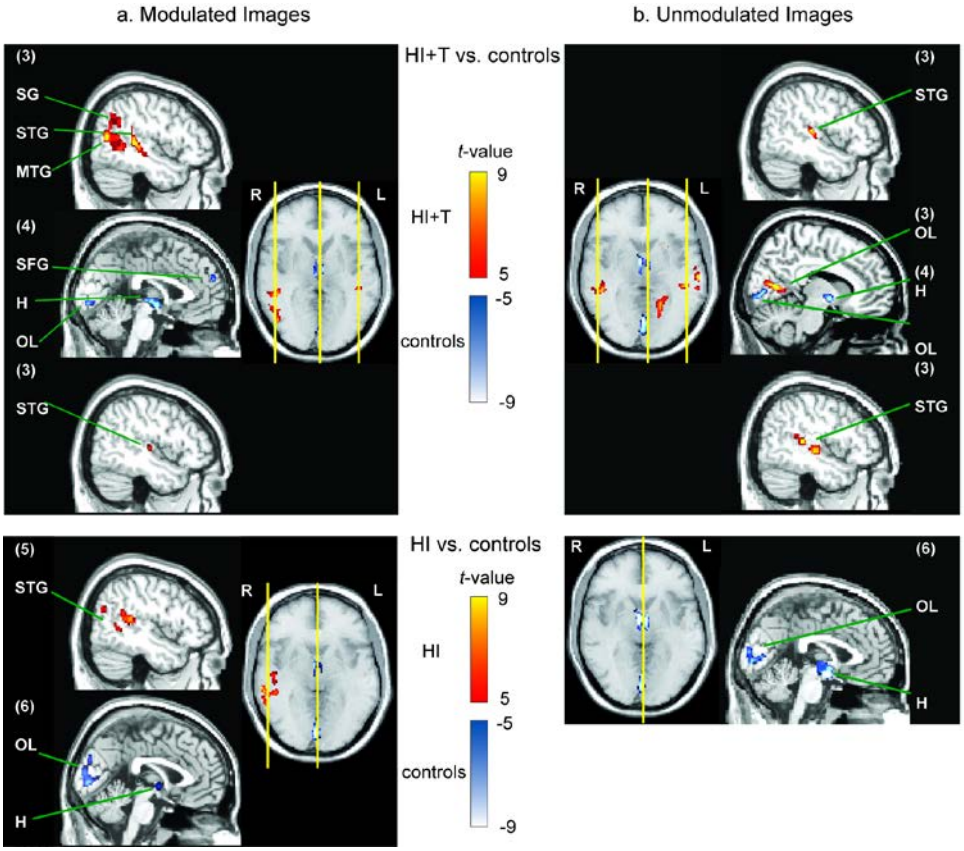

Figure 3.2. Statistical parametric maps of whole-brain voxel-by-voxel VBM analyses for (a) modulated gray matter images and (b) unmodulated gray matter images. GM increases (red-yellow) and decreases (blue-white) are shown for the hearing-impaired subjects with tinnitus (HI+T) and those without tinnitus (HI) relative to the controls (no significant differences were found between the HI+T and HI groups). The threshold was set at $p < 0.05$ FWE corrected at voxel-level. Numbers between brackets refer to the contrasts and results in Table 3.3. SG: supramarginal gyrus; STG: superior temporal gyrus; MTG: middle temporal gyrus; SFG: superior frontal gyrus; H: hypothalamus; OL: occipital lobe.

Table 3.3. Voxel-by-voxel comparisons of GM volume obtained from the modulated images and GM volume obtained from the unmodulated images with a threshold of $p < 0.05$ FWE corrected, and an extent threshold (k) of 50 contiguous voxels. Local maxima from the different contrasts and the corresponding Brodmann Areas (BA) for the clusters are specified in the table. Left hemisphere: $x < 0$; right hemisphere: $x > 0$; HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired; C: controls.

Voxel-by-Voxel Comparisons: Two-Sample *t*-tests, $p < 0.05$ FWE at the Cluster and Voxel Levels; k = 50						
	MNI Coordinates (x y z)			**Z Score**	**Cluster size (voxels)**	**BA**
MODULATED IMAGES						
1) HI+T > C						
Superior Temporal Gyrus, Left	-45	-34	11	5.28	76	41
Superior Temporal Gyrus, Right	53	-43	14	6.27	1069	22/41
Middle Temporal Gyrus, Right	57	-53	6	5.09	151	22
	49	-69	13	5.67	933	39
2) HI+T < C						
Superior Frontal Gyrus, Left	-7	60	-17	5.12	59	11
	-13	62	19	5.12	71	10
	-16	47	39	5.4	167	8/9
Occipital Lobe, Right	1	-84	-3	5.46	198	17/18
Hypothalamus, Right	4	-5	11	6.1	987	-
Caudate Nucleus, Right	1	3	3	5.69	64	-
3) HI > C						
Superior Temporal Gyrus, Right	62	-35	9	5.1	166	22/42
	52	-41	13	5.08	107	22/40
Middle Temporal Gyrus, Right	52	-17	9	5.51	56	22
4) HI < C						
Occipital Lobe, Right	2	-88	8	5.7	1372	17/18
UNMODULATED IMAGES						
1) HI+T > C						
Superior Temporal Gyrus, Left	-47	-33	10	6.15	159	41
	-61	-24	3	5.66	119	22
	-59	-38	14	5.83	230	
Superior Temporal Gyrus, Right	51	-40	13	5.55	459	22/41
Occipital Lobe, Left	-18	-56	-1	5.85	573	18/19/30
2) HI+T < C						
Occipital Lobe, Right	1	-72	4	6.24	958	18
Hypothalamus, Left	-4	-9	-6	5.87	350	-
Hypothalamus, Right	5	-5	-10	6.64	544	-
3) HI < C						
Hypothalamus, Right	5	-6	-8	5.82	260	-

3.3.3 ROI analyses

Per subject, the mean GM-M and GM-U for each ROI, scaled by the whole-brain GM volumes, were calculated. In total, 88 ROIs (39 BAs on the left and right side, four auditory nuclei on the left and the right side, and the left and right cerebellum) were considered. The results of the one-way ANCOVAs that were performed on the BAs are shown in **Figure 3.3**. Only the BAs that showed a significant difference between the three groups are shaded ($p < 0.05$, FDR corrected). Significant scaled GM-M (**Figure 3.3a**) and GM-U (**Figure 3.3b**) differences were present in the temporal lobe, the limbic/entorhinal cortex, the frontal lobe and the occipito-parietal lobe. No significant GM-M or GM-U difference between the groups was found for the subcortical auditory nuclei and the cerebellum.

For all BAs for which GM-M or GM-U was significantly different between the groups (i.e. for the shaded areas in **Figure 3.3**), **Figure 3.4a** and **3.4b** indicate GM-M and GM-U differences for the HI+T and the HI groups compared to the control group in the left hemisphere and right hemisphere respectively. Furthermore, these figures detail which pairwise comparisons were significant. For both the HI+T group and the HI group, similar GM-M and GM-U effects relative to the controls were found: increases were mainly observed in the temporal lobe and the limbic/entorhinal cortex; decreases were mainly found in the frontal and occipito-parietal lobes. In the following, we will highlight the BAs for which the analyses of GM-M and GM-U gave consistent results.

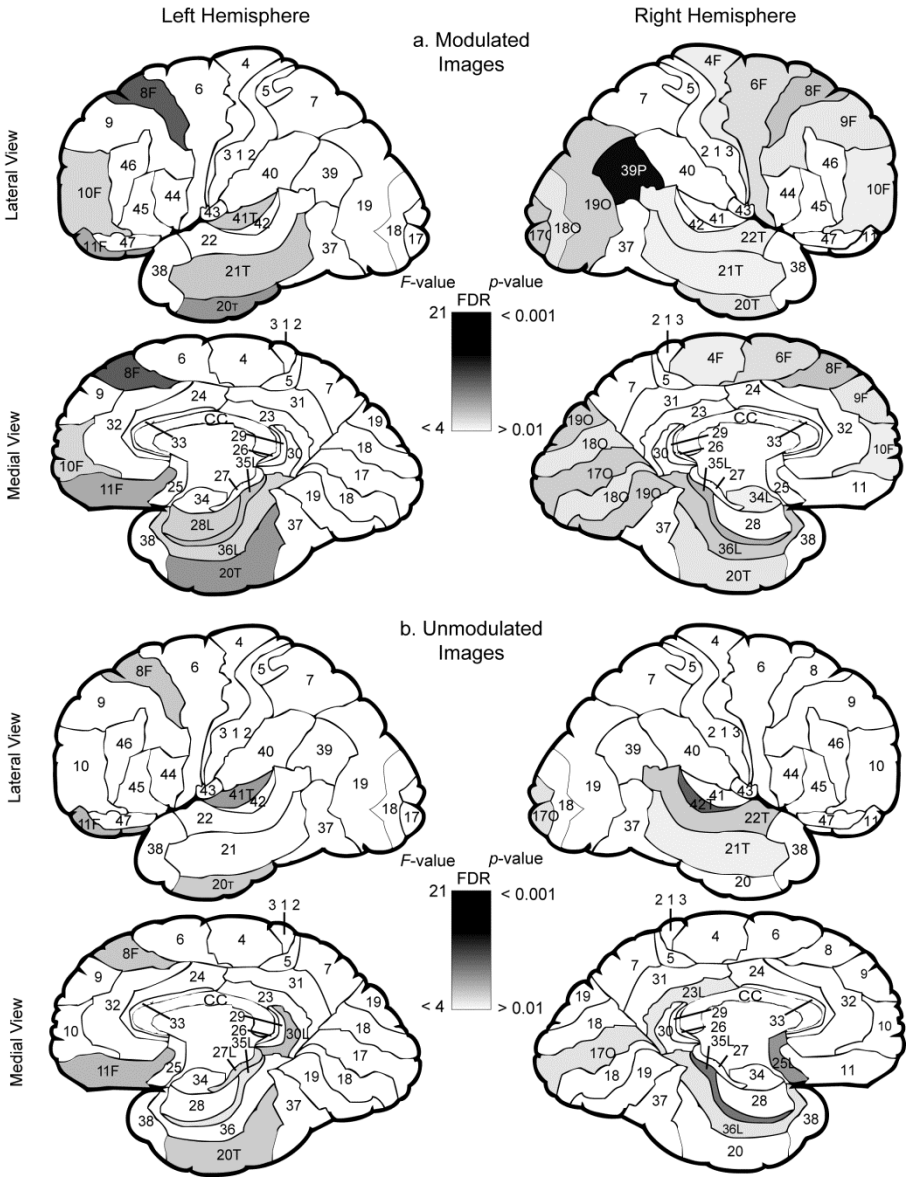

Figure 3.3. Differences between the three subject groups in (a) modulated gray matter images and (b) unmodulated gray matter images. The gray shading indicates the *F*-value obtained from an ANCOVA analysis of the average gray matter signal per Brodmann area, where age was an incorporated covariate. Only the Brodmann areas with a significant *F*-value, FDR corrected, are shaded. CC: corpus callosum; F: frontal lobe; L: limbic lobe; O: occipital lobe; P: parietal lobe; T: temporal lobe.

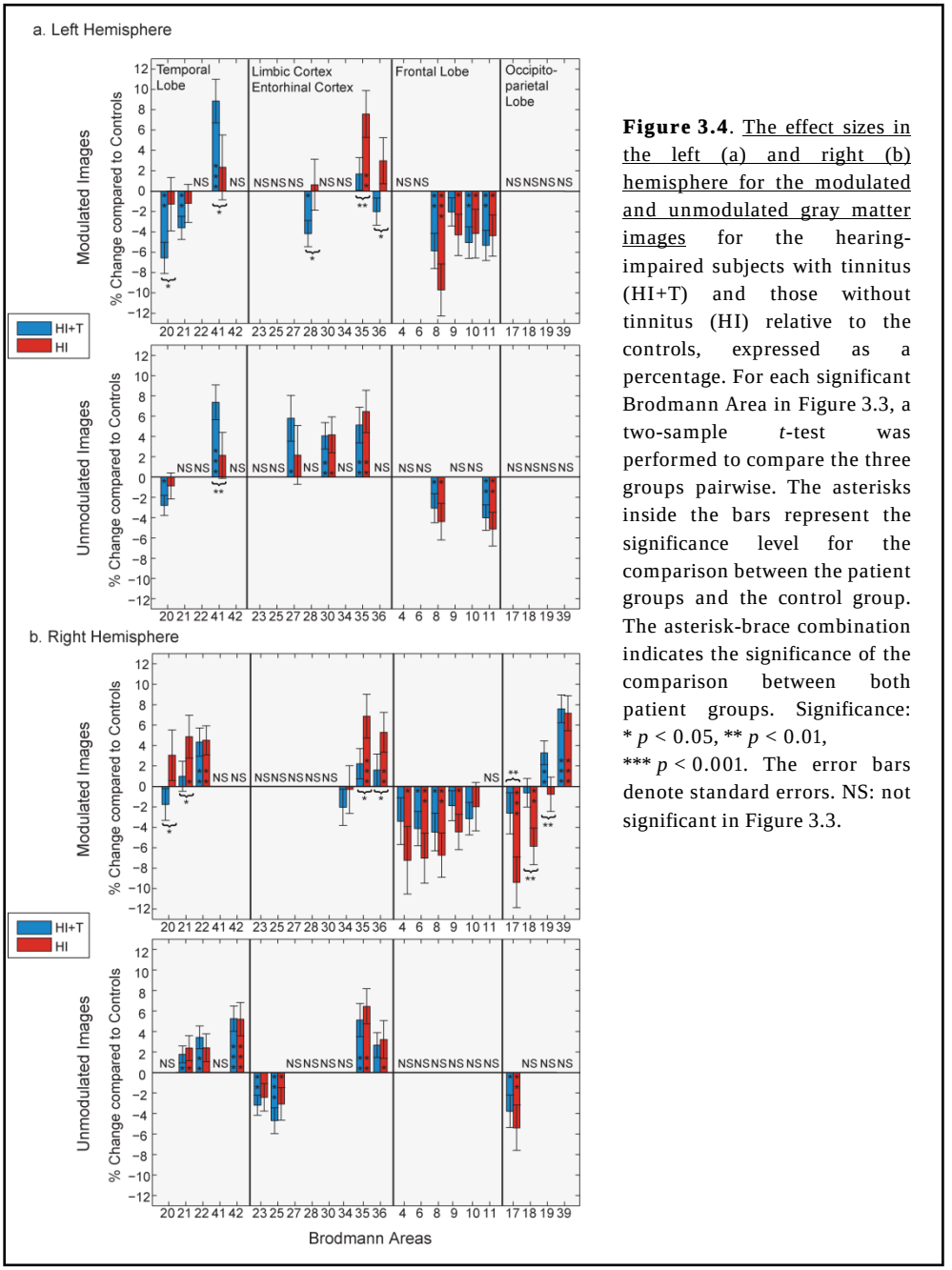

Figure 3.4. The effect sizes in the left (a) and right (b) hemisphere for the modulated and unmodulated gray matter images for the hearing-impaired subjects with tinnitus (HI+T) and those without tinnitus (HI) relative to the controls, expressed as a percentage. For each significant Brodmann Area in Figure 3.3, a two-sample *t*-test was performed to compare the three groups pairwise. The asterisks inside the bars represent the significance level for the comparison between the patient groups and the control group. The asterisk-brace combination indicates the significance of the comparison between both patient groups. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The error bars denote standard errors. NS: not significant in Figure 3.3.

In left temporal BA 41 (primary auditory cortex), a significant difference between the groups was found for both GM-M ($F = 7.47$, $p = 0.0012$) and GM-U ($F = 10.63$, $p = 9.8\times10^{-5}$). For the HI+T group, an increase in both measures was present relative to the HI group as well as the control group. This increase of GM was the largest effect we observed across all BAs for the HI+T group. To test whether this increase could be attributed to tinnitus as assessed by the THI questionnaire, an ANCOVA regression model was set up that included either GM-M or GM-U of the left BA 41 as the response variable, age and HQ score as additional explanatory variables of no interest, and THI score as the explanatory variable of interest. The results showed a significant effect of THI scores on both the GM-M ($F = 4.18$, $p = 0.045$) and GM-U ($F = 4.40$, $p = 0.040$) measures in the left BA 41. Therefore, we may conclude that the GM increase in the left primary auditory cortex can be attributed to tinnitus.

Moreover, significant GM-M ($F = 4.84$, $p = 0.011$) and GM-U ($F = 7.34$, $p = 0.0013$) differences between the groups were found for the right temporal BA 22 (auditory association cortex). The increases relative to the control group were significant for both patient groups. No significant differences between the HI+T and the HI groups were observed.

Furthermore, in temporal BA 20 (inferior temporal area), significant group differences were found in the left (GM-M: $F = 10.92$, $p = 7.9\times10^{-5}$; GM-U: $F = 7.39$, $p = 0.0013$) and right hemisphere (GM-M: $F = 5.64$, $p = 0.0054$). In both hemispheres, significant GM-M decreases were found for the HI+T group relative to the HI group. In the left hemisphere, the HI+T group showed a significant GM-M decrease relative to the control group as well. For GM-U, only a significant decrease in the left hemisphere for the HI+T group relative to the control group was observed.

In the limbic cortex, group differences were observed in the left and right limbic BA 35 (overlaps with the parahippocampal gyrus), both for GM-M (left: $F = 7.85$, $p = 8.7\times10^{-4}$; right: $F = 8.93$, $p = 3.6\times10^{-4}$) and GM-U (left: $F = 5.43$, $p = 0.0065$; right: $F = 13.14$, $p = 1.5\times10^{-5}$). For the modulated images, the GM increase was bilaterally significant for the HI group relative to both the HI+T group and the control group. For the unmodulated images, bilateral significant increases relative to the control group were observed in both patient groups. No significant difference between the patient groups was observed.

Significant group differences were found in the limbic BA 36 (ectorhinal area) as well. For the GM-M images, this effect was found in both the left ($F = 6.62$, $p = 0.0024$) and the right ($F = 7.31$, $p = 0.0013$) hemisphere. In both hemispheres, a significant increase for the HI group relative to the HI+T group was observed. In

the right hemisphere, the HI group had significantly more GM than the control group as well. For the GM-U images, the significant group effect was only found in the right hemisphere ($F = 5.73$, $p = 0.0051$). Both patient groups showed an increase relative to the control group, but this increase was only significant for the HI group.

In the frontal lobe, significant group differences were found in BAs 8, 9 and 11. In the left hemisphere, the group differences in BA 8 (premotor cortex) were found for both the GM-M ($F = 14.83$, $p = 4.7\times10^{-6}$) and the GM-U ($F = 7.63$, $p = 0.0010$) images. Significant decreases for both hearing-impaired groups relative to the control group were found in both measures. In the right hemisphere, the effect was only found for the GM-M images ($F = 7.86$, $p = 8.6\times10^{-4}$). Yet again, significant decreases for both hearing-impaired groups relative to the control group were found. For none of the comparisons, significant differences between both patient groups were detected.

In BA 9 (part of prefrontal cortex), significant group differences were revealed for the GM-M images in both hemispheres (left: $F = 4.56$, $p = 0.014$; right: $F = 5.47$, $p = 0.0063$). The patient groups showed GM decreases relative to the control group, but only the differences between the HI and the control group were significant. No significant differences between the HI+T and HI groups were noticed.

In BA 11 (part of prefrontal cortex), significant group differences were revealed in the left hemisphere for both the GM-M ($F = 9.68$, $p = 2.0\times10^{-4}$) and GM-U ($F = 8.61$, $p = 4.7\times10^{-4}$) images. For both measures, significant decreases in the hearing-impaired groups compared to the control group were found. No significant difference between both hearing-impaired groups could be detected.

3.4 Discussion

The aim of the present study was to investigate GM differences in patients with hearing loss and tinnitus, patients with hearing loss alone and normal-hearing controls, in order to specifically assess the effect of tinnitus relative to the effect of hearing loss alone. Both voxel-by-voxel comparisons and ROI analyses were performed. Both voxel-by-voxel comparisons and ROI analyses on the BAs showed a number of localized GM differences between the subject groups. We found GM increases in the superior and medial temporal lobes of the patient groups, with the most prominent effect being an increase of GM in the left primary auditory cortex (BA 41) of the tinnitus subjects. In addition, both tinnitus and hearing loss were associated with increases in entorhinal/limbic areas, and mainly decreases in frontal and occipital brain areas. These results show that both hearing loss and tinnitus are related to wide-spread neuroanatomical GM differences in the brain, as has been similarly observed in a number of previous studies. Additionally, they suggest a specific role of the left primary auditory cortex in tinnitus.

For all analyses, we chose to include age as an explanatory variable of no interest. This choice allows us to correct for the large effect that age has on GM in the brain (Good et al., 2001). At the same time, including this covariate corrects for the correlation between age and hearing loss, and for the age differences between the groups. As a result, the observed effects were not associated with age.

To date, five studies have investigated GM alterations in tinnitus patients (Mühlau et al., 2006; Landgrebe et al., 2009; Husain et al., 2011; Leaver et al., 2011; Schneider et al., 2009). An overview of the results is given in **Table 3.1**. Although each study did find differences in the auditory and/or limbic regions, confirming the involvement of both systems in the pathophysiology of tinnitus, large differences between the results of the studies could be noticed. To compare our study to the previous studies, the reported coordinates of the peak voxels in the previous studies were used to identify the corresponding BA by using the WFU_pickatlas (Maldjian et al., 2003). As will be discussed below, our study reproduced some of the effects found in the previous studies, but many differences were noticed as well.

A first obvious difference between our study and the previous VBM studies relates to the number of brain areas for which differences were observed between the subject groups.

Our study yielded extensive brain areas with significant differences, while previous tinnitus studies typically only found few. This difference may be due to several causes. Firstly, the relatively large number of subjects participating in our study presumably lead to an increased statistical power. Secondly, our two hearing-impaired groups were well matched in terms of hearing loss such that the primary difference between these two groups was the presence or absence of tinnitus. These two factors enabled us to sensitively and specifically look for effects of hearing impairment and tinnitus. Moreover, we corrected for the possible variance of gray matter due to the range of ages in our subject groups by incorporating age as a covariate in the ANCOVAs that were used to test for differences between groups. Finally, we applied a ROI analysis based on relatively large Brodmann areas. This analysis was neither restricted to some particular brain areas based on previous studies nor derived from outcomes of voxel-by-voxel comparisons. Rather, the entire cortex was piecewise explored. The added value of applying large ROIs consists of the possibility to detect weak but extensive changes of gray matter that may remain undetected in the whole-brain analysis because of the multiple comparisons across voxels. Together these four factors may have led to an increased sensitivity to alterations of gray matter.

A major finding of our study is that mild to moderate hearing loss, irrespective of tinnitus, causes GM decreases and increases in cortical regions associated with auditory processing. To our knowledge, we are the first to report GM differences in the auditory cortex in whole-brain voxel-by-voxel analyses due to hearing loss. Remarkably, no differences between the HI+T and HI groups could be found in the whole-brain voxel-by-voxel analysis. Only when applying ROIs, GM differences related to hearing loss and tinnitus respectively could be disentangled. This suggests that the differences between both hearing-impaired groups were too small to be detected in the whole-brain analysis.

Based on previous studies, it is not surprising that hearing loss causes GM changes. Although previous studies always reported GM decreases in the auditory cortex (Schneider et al., 2009; Husain et al., 2011), we found GM increases for both patient groups relative to the control group. The increases in BA 22, found in both the whole-brain and the ROI analyses, may be related to the role of BA 22 in semantic memory (Dalla Barba et al., 1998; Lee et al., 2002). It is likely that hearing-impaired subjects will miss a substantial proportion of phonemes in a stream of speech sounds. Therefore, it is conceivable that they rely more heavily on contextual information from semantic memory in order to maintain normal communication. The increased use of this associative auditory function may have increased the GM volume in the auditory association area BA 22.

The largest difference between the subjects with and without tinnitus concerned an increase in the GM volume in the left primary auditory cortex BA 41 of the tinnitus patients. An important function of the primary auditory cortex covers the processing of simple auditory stimuli, such as pure tones and noise (Mirz et al., 1999). Since tinnitus is most experienced as (a combination of) tones or noise, it may be that continuously hearing an internal sound is related to an increased volume in the primary auditory cortex, although the left lateralization is hard to interpret. Remarkably, our result is opposite to the result of Schneider et al. (2009), who found GM reductions in primary auditory cortex in tinnitus subjects with normal-hearing thresholds. It is noteworthy that none of the other previous studies showed an effect in the primary auditory cortex. This may be due to the methodological differences in data analysis discussed above, because we were only able to detect the difference between both hearing-impaired groups by applying the ROI analyses on the BAs including BA 41. In addition, ten of our tinnitus subjects suffered from moderate to severe tinnitus (THI > 38), which may have exacerbated some of the outcomes. Note that a specific role of the left auditory cortex has also been inferred from PET-imaging (Lockwood et al., 1998; Langguth et al., 2006) and transcranial magnetic stimulation (TMS) studies (Burger et al., 2011).

In addition to the temporal lobe, we found GM differences in the limbic/entorhinal cortex. For the voxel-by-voxel comparisons, GM decreases were found in the hypothalamus for both hearing-impaired groups relative to the control group. Furthermore, the hearing-impaired groups showed GM increases in the parahippocampal gyrus (BA 35). Differences between the groups were found in ectorhinal area (BA 36) as well. Both areas are involved in emotional processing. Comparing the GM-M and GM-U outcomes suggests a differential effect of hearing loss and tinnitus. Landgrebe et al. (2009) found a GM decrease for the tinnitus subjects compared to the normal-hearing controls at coordinates corresponding to BA 28, and attributed these to a decrease in GM in the hippocampus. For this area, we only found GM-M differences in the left hemisphere. Our results are however consistent with the finding of Landgrebe et al. (2009): a GM decrease for the tinnitus group relative to the controls. In addition, we found this effect for the tinnitus subjects compared to the hearing-impaired subjects as well. GM decreases in the left hippocampus have been hypothesized to be involved in the pathophysiology of depression (de Geus et al., 2007) and insomnia (Riemann et al., 2007). Both symptoms occur frequently in tinnitus patients. Similar to Landgrebe et al. (2009), because none of our tinnitus subjects satisfied the diagnostic criteria for major depression or suffered from severe insomnia, it seems unlikely that the hippocampal changes are merely due to the incidence of co-morbid depression or insomnia. A negative influence of high intensity noise exposure on hippocampal

neurogenesis in adult rats was already demonstrated by Kraus et al. (2010). The results of both our study and the study of Landgrebe et al. (2009) suggest a GM decrease in the hippocampus related to tinnitus rather than to hearing loss alone. The observation that numerous parts of the limbic system show alterations for both hearing-impaired groups is indicative of the involvement of the emotional brain in tinnitus, confirming previous studies (Jastreboff, 1990; Leaver et al., 2011; Rauschecker et al., 2010; De Ridder et al., 2011), but suggests an emotional response to hearing loss as well.

Two previous studies found significant GM differences in the subcallosal area, overlapping with BA 25: Mühlau et al. (2006) and Leaver et al. (2011) found GM decreases for their tinnitus groups compared to their controls, suggesting that the observed differences are related to tinnitus. We only found a GM-U effect in the right BA 25 where the significant GM reductions apply for both our hearing-impaired groups relative to our control group. The findings of Mühlau et al. (2006), Leaver et al. (2011) and us suggest that the subcallosal area is involved in both hearing loss and tinnitus.

Differences in the frontal cortex between the groups were found as well. Frontal areas are associated with working memory and higher cognitive processes rather than with sensory processing. The differences mainly concerned decreases for both hearing-impaired groups relative to the normal-hearing control group. This outcome confirms the finding of Husain et al. (2011) who observed significant decreases in the superior and medial frontal gyri for their hearing-impaired group relative to their normal-hearing control group. In addition, they found significant decreases for their hearing-impaired group compared to their hearing-impaired tinnitus group. Husain et al. attributed the GM decreases to peripheral hearing loss. Our results support their idea.

Our study suffered from the limitation that no audiometric data were available from our control group. Given the age of the control group (58±6 years), there is a possibility that the subjects had an unnoticed hearing loss. However, from literature we know that the average audiogram of individuals in their fifties is near-normal (PTA ≤ 30 dB HL) (Cruickshanks et al., 1998; Demeester et al., 2009). Furthermore, our control subjects were known not to wear hearing aids. Altogether, we assume that our control subjects were appropriate.

Although our two hearing-impaired groups were well matched in terms of hearing loss such that the primary difference between these two groups was the presence or absence of tinnitus, we cannot definitively claim that the GM differences found may only be due to tinnitus. Considering the significant difference in HQ scores, the between-group differences seen here could be due to

hyperacusis as well. Hyperacusis as such was not an inclusion criterion, but a subject characteristic. It is very difficult to disentangle the effects of hyperacusis and tinnitus since these characteristics were correlated (R = 0.59). For our largest effect found in the left BA 41, we showed that the increase could be attributed to tinnitus as assessed by the THI questionnaire. A larger study including a tinnitus subgroup suffering from hyperacusis and an additional tinnitus subgroup without hyperacusis would be necessary to clearly distinguish the effects of hyperacusis from those of tinnitus.

Would it be possible to use VBM to distinguish between hearing-impaired subjects with and without tinnitus? If so, a VBM analysis would be of potential clinical use in the diagnosis of tinnitus. By considering the Receiver Operating Characteristic (ROC) curve of the GM value of the left primary auditory cortex (BA 41), tinnitus subjects could be identified with approximately 80% sensitivity and 70-80% specificity (**Figure 3.5**). The areas under the curves (AUCs) for GM-M and GM-U are 0.75 and 0.79 respectively, indicating that a randomly selected individual from the tinnitus group has a GM value larger than that for a randomly chosen individual from the subjects without tinnitus in 75%, respectively 79%, of the time. The specificity and selectivity of this analysis are currently insufficient to serve as a practical test in clinical diagnosis. However, it can be seen as a stepping stone to a more extensive analysis involving other brain areas and (possibly) other brain imaging techniques. The further development of VBM as a diagnostic tool will require an independent validation in a new group of subjects. To enhance the interpretation of the GM ROC curves, additional ROC curves for d' were added to the figure. The sensitivity index d' provides the separation between the means of the distribution under noise-alone conditions (healthy subjects) and its distribution under signal-alone conditions (tinnitus subjects), under the assumption that both these distributions are normal with the same standard deviation (Oliver et al., 2008).

Based on our results, it is impossible to draw conclusions on the causal relation between GM differences, hearing loss and tinnitus. In the case of hearing loss, it seems unlikely that abnormalities in the brain cause peripheral sensorineural hearing loss. Far more likely, the GM differences are a consequence of the hearing loss. For instance, a decrease of sensory input may be conceived to lead to deafferentation, neuronal cell degeneration, and – ultimately – cortical atrophy. In the case of tinnitus, it is more difficult to speculate on its causal relation to GM properties. The GM increase in the left primary auditory cortex of tinnitus subjects could represent a pre-existing vulnerability to develop tinnitus in response to sensorineural hearing loss. Alternatively, the GM increase may be caused as a consequence of ongoing neural activity (Husain et al., 2011). Understanding the

causal relation between tinnitus and changes in GM will be an important next step in understanding the pathophysiology of tinnitus.

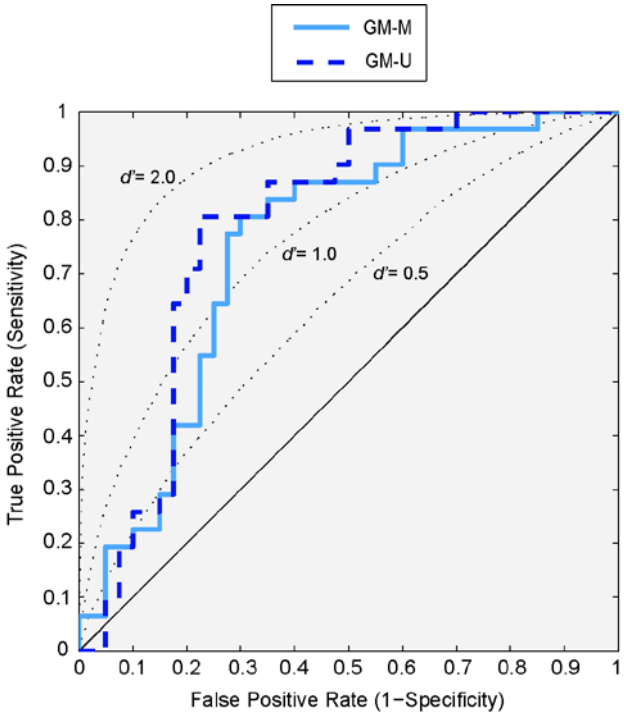

Figure 3.5. Receiver Operating Characteristic curves for GM-M and GM-U in the left primary auditory cortex. The area under the curve of GM-M equals 0.75 and of GM-U equals 0.79 (both significantly different from 0.5: $p < 0.001$). Tinnitus subjects could be identified with approximately 80% sensitivity and 70-80% specificity. The dotted curves indicate the ROC curves for $d' = 0.5$, $d' = 1.0$ and $d' = 2.0$ respectively.

3.5 Conclusion

In our study, the hearing-impaired groups (both with and without tinnitus) showed mainly GM increases in the temporal and limbic areas, and decreases in the frontal and occipital areas, compared to the control group. The most significant effect was found in the left primary auditory cortex, where tinnitus was associated with an increase in gray matter.

ACKNOWLEDGEMENTS

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Chapter 4

Tinnitus-Related Dissociation between Cortical and Subcortical Neural Activity in Humans with Mild to Moderate Sensorineural Hearing Loss

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ABSTRACT

Tinnitus is a phantom sound percept that is strongly associated with peripheral hearing loss. However, only a portion of hearing-impaired subjects develops tinnitus. This may be based on differences in the function of the brain between those subjects that develop tinnitus and those that do not. In this study, cortical and sub-cortical sound-evoked brain responses in 34 hearing-impaired chronic tinnitus patients and 19 hearing level-matched controls were studied using 3-T functional magnetic resonance imaging (fMRI). Auditory stimuli were presented to either the left or the right ear at levels of 30-90 dB SPL. We extracted neural activation as a function of sound intensity in eight auditory regions (left and right auditory cortices, medial geniculate bodies, inferior colliculi and cochlear nuclei), the cerebellum and a cinguloparietal region. The activation correlated positively with the stimulus intensity, and negatively with the hearing threshold. We found no differences between both hearing-impaired groups in terms of the magnitude and lateralization of the sound-evoked responses, except for the left medial geniculate body and right cochlear nucleus where activation levels were elevated in the tinnitus subjects. We observed significantly reduced functional connectivity between the inferior colliculi and the auditory cortices in tinnitus patients compared to controls, both for ipsilateral and contralateral connections. Our results indicate a failure of thalamic gating in the development of tinnitus.

4.1 Introduction

Tinnitus is a poorly understood auditory percept in the absence of an external stimulus and is typically associated with hearing loss. It is a common disorder with prevalence estimates ranging from 7 to 20% (Hoffman and Reed, 2004). Approximately 40% of the tinnitus patients also suffer from hyperacusis, a diminished tolerance to ordinary environmental sounds (Baguley, 2003). Most patients with chronic tinnitus are continuously aware of the tinnitus percept, but are able to cope effectively with the disturbance. However, for some patients the tinnitus is more than a trivial annoyance resulting in feelings of desperation and even suicidal thoughts (Dobie, 2003).

An important role in the generation of tinnitus is currently attributed to mechanisms in the central auditory system. Animal studies have shown that manipulations that are known to be the source of tinnitus in humans (e.g. noise trauma), cause increased spontaneous neural activity or changes in neural synchrony in auditory brain structures (Noreña and Eggermont, 2003; Seki and Eggermont, 2003). A number of functional magnetic resonance imaging (fMRI) studies have investigated the neural correlates of tinnitus in humans (for a review, see Lanting et al., 2009). Functional MRI is unable to register increased spontaneous activity. Consequently, the fMRI studies applied sound stimuli to probe abnormal sound processing in the brain of tinnitus patients. Measuring changes in hemodynamics as a response to sound in tinnitus sufferers revealed increased activation in the inferior colliculus compared to controls (Lanting et al., 2008; Melcher et al., 2009), although this may have been associated with hyperacusis rather than with tinnitus (Gu et al., 2010). In contrast to the brainstem, elevated sound-evoked auditory cortex activation can be attributed to tinnitus (Gu et al., 2010). These three studies all show neural correlates of tinnitus in clinically normal-hearing subjects.

The majority of tinnitus patients, however, has a significant hearing loss. As was shown in numerous animal studies, hearing loss is associated with adaptation in the central auditory system, which is likely to be related to tinnitus (for a review, see Eggermont, 2001). Since tinnitus does not develop in all hearing-impaired individuals, it must be assumed that these adaptions are different between those that develop tinnitus and those that do not. The aim of this study was to investigate this hypothesis in subjects with a mild to moderate sensorineural hearing loss. Two relatively large subject groups were enrolled: a hearing-impaired group without tinnitus and a matched hearing-impaired group suffering from tinnitus. We used fMRI to measure sound-evoked responses throughout the brain,

primarily focusing on the auditory system. Differences between both groups were investigated with respect to the magnitude of brain responses, their lateralization, and the functional connectivity patterns between brain regions.

4.2 Materials and methods

4.2.1 Subjects

This study included data collected from two groups of patients. The patients were recruited at the University Medical Center Groningen (UMCG) and via hearing aid dispensers in Groningen, the Netherlands. The first group comprised 19 hearing-impaired subjects (HI group). The second group comprised 34 subjects with a hearing impairment suffering from tinnitus (HI+T group). Pure-tone audiometry was performed with a clinical audiometer using six different octave frequencies (0.25, 0.5, 1, 2, 4 and 8 kHz). For all subjects, the pure-tone average (PTA) hearing threshold at the octave frequencies of 1, 2 and 4 kHz satisfied $30 \leq \text{PTA} \leq 60$ dB in both ears.

Further details of the subjects were obtained by two questionnaires that examine handedness and tinnitus handicap, respectively. To assess handedness, a translated version of the Edinburgh Inventory (Oldfield, 1971) was completed by all subjects. In the tinnitus subjects only, tinnitus handicap was assessed by a Dutch translation of the Tinnitus Handicap Inventory (THI), a self-reported tinnitus handicap questionnaire (Newman et al., 1996). None of the subjects had any major medical, neurological or psychiatric history.

This study was approved by the local medical ethics committee. All subjects were informed about the purpose of the study before giving their written consent in accordance with the Dutch legislation.

4.2.2 Data acquisition

The imaging experiments were performed using a 3-T MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands), which was equipped with an eight-channel phased-array (SENSE) head coil. The functional scans consisted of 2200-ms single-shot T_2*-sensitive echo planar imaging (EPI) sequences with 41 3-mm thick slices (TR 10 s; TE 22 ms, flip-angle 80°; voxel size $1.75 \times 1.95 \times 3$ mm^3; field of view $224 \times 224 \times 123$ mm^3) and were acquired using a near-coronal

orientation, aligned to the brainstem when viewed on a midsagittal cross-section. Each image volume enclosed left and right cochlear nuclei (CN), inferior colliculi (IC), medial geniculate bodies (MGB) and auditory cortices (AC). The influence of acoustic scanner noise was reduced using a sparse sampling strategy (Hall et al., 1999; Langers et al., 2005). Auditory stimuli were presented during a 7.8-s gap of scanner silence between two successive acquisitions. For each subject, three runs of 73 acquisitions were performed. Additional start-up scans that were included to achieve magnetization equilibrium and to trigger the start of the stimulus were excluded from analysis. In addition, a 3-dimensional high-resolution T_1-weighted fast-field echo scan (TR 9 ms; TE 3.50 ms; flip-angle 8°; voxel size 1 x 1 x 1 mm^3; field of view 256 × 256 × 170 mm^3) was acquired with the same orientation as the functional scans to serve as an anatomical reference.

4.2.3 Acoustic stimulation and scanning paradigm

Auditory stimuli were delivered by an MR-compatible electrodynamic system (MR Confon GmbH, Magdeburg, Germany; Baumgart et al. 1998), connected to a PC setup equipped with a digital-to-analog converter controlled by Labview 6.1 (National Instruments 6052E, National Instruments Corporation, Austin, TX, USA). The stimuli consisted of dynamic ripples (Langers et al., 2003). The spectrum of a dynamic ripple is based on pink noise, but contains temporal and spectral modulations. The stimuli comprised frequency components between 125 and 8000 Hz, with a spectral modulation density of one cycle per octave, a temporal modulation frequency of two cycles per second, and a modulation amplitude of 80%. These stimuli were chosen for their potency to induce a robust sound-evoked responses in the auditory pathway (Langers et al., 2003; Lanting et al., 2008; Lanting et al., 2010).

During the gaps of scanner silence between two successive acquisitions, auditory stimuli were presented to the left (L) or the right (R) ear at either 30, 50, 70 or 90 dB SPL (L_{30}, L_{50}, L_{70}, L_{90}; R_{30}, R_{50}, R_{70} or R_{90}). In addition, a silent baseline condition was included. The stimuli were presented in a fixed pseudo-random order in each functional run. Per run, the silent condition was presented nine times and all the stimulus conditions were presented eight times each. During the functional scans, the subjects were instructed to register whether they perceived an audible stimulus using a button box: whenever they perceived an audible stimulus in the left or right ear, they pressed one of two corresponding buttons with their right thumb. This task was imposed in order to promote and monitor that the subject paid attention to the presented sound stimuli.

4.2.4 Data processing and linear regression analysis

The images were analyzed using SPM8 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) and MatLab 7.1 (The Mathworks Inc., Natick, MA). The functional images were first corrected for motion using realignment on the basis of 3-D rigid body transformations. The T_1-weighted high-resolution anatomical images were spatially co-registered to the functional images, and all images were normalized into Montreal Neurological Institute (MNI) stereotaxic space. To improve the signal-to-noise ratio, the functional data were spatially smoothed using an isotropic Gaussian kernel with a full width at half maximum of 4 mm. In order to express the signal measures in percentage signal change, a logarithmic transformation was carried out (Langers and van Dijk, 2011). The functional images were interpolated to voxel dimensions of 2.0 x 2.0 x 2.0 mm^3.

Per subject, a general linear model was set up to analyze the relative contribution of each condition to the measured response. The model included one covariate of interest for each of the eight stimulus conditions, the realignment parameters, as well as constant and linear terms to model the baseline and drift within each run. An omnibus *F*-test, including contrasts of all individual stimulus conditions relative to baseline, was assessed in each voxel to detect the combined effect of all sound stimuli. The confounds (i.e. baseline, drift and realignment) that were estimated were subtracted from the preprocessed functional acquisitions for the purpose of subsequent connectivity analyses.

The contrast images of the eight sound conditions of interest, relative to the silent condition, were entered in a second-level random-effects analysis based on a flexible factorial design with factors for group (i.e. HI+T and HI), subject, and stimulus condition. Significant responses to all sound conditions across all subjects were detected by means of an *F*-test.

4.2.5 ROI definitions and functional connectivity

Eight regions of interest (ROIs) were defined, comprising both left and right AC, MGB, IC and CN. The ROIs of the left and right AC, MGB and IC were defined by means of the outcomes of the second-level random-effects analysis (see Results). The AC ROI comprised all supra-threshold voxels in the temporal lobe ($F_{7,357} > 6.53$; $p < 0.05$ corrected for family-wise errors). The MGB and IC ROIs were drawn to include a cluster of supra-threshold voxels around the correspondingly localized activation maxima. Because no activation of the left or right CN was found, these ROIs were defined as a sphere with a radius of 5 mm positioned at MNI coordinates (±10, −38, −45) consistent with a previous publication of our group (Boyen et al., 2012).

Next, functional connectivity analysis was performed. Functional connectivity examines the correlations among activity in different brain areas (Friston, 1994; Smith et al., 2009). Time courses were determined for all auditory ROIs by averaging the preprocessed hemodynamic signals of all voxels in the respective ROI and concatenating them across all subjects. For each ROI, a functional connectivity map was derived by calculating the Pearson correlation coefficients *R* between the time course of the respective ROI and the time courses of all other voxels in the brain.

Based on the group connectivity maps (see 3.3 Functional connectivity maps), two more ROIs were included: the cerebellum (CER) and a cinguloparietal region (CPR). The ROI of the CER consisted of the anterior and posterior cerebellar lobes. The CPR was comprised of the bilateral cingulate cortex, the primary somatosensory cortex (Brodmann Areas (BAs) 1, 2 and 3), the primary motor cortex (BA 4) and the supplementary motor area (BA 6). Both ROIs (CER and CPR) were defined according to the WFU_pickatlas (Maldjian et al., 2003).

4.2.6 Response amplitudes and lateralization of Regions-of-Interest

For each subject, the 10% most strongly responding voxels within each ROI according to the individual *F*-test were selected (see **Table 4.3**), and per condition the signal change relative to baseline was averaged over these voxels. Statistical analyses were performed using a repeated measures ANCOVA model for each ROI separately. In addition to the factors for group, subject and stimulus condition that were included in the second-level random-effects model already, this model included the subject's PTA hearing loss in the ear of stimulus presentation as

covariate. The main effects of group and stimulus condition and the interaction between these two factors were determined. In addition, we tested whether neural activation in response to the stimuli co-varied with the amount of hearing loss in the ear of presentation.

For each ROI of each subject, the average response to all stimuli presented to the left (*L*) and the right (*R*) ear, respectively, was calculated. The values obtained were used to calculate a lateralization index

$$\mathrm{LI} = \frac{L - R}{|L| + |R|}$$

For positive responses, a value of +1 indicates a response to stimuli presented at the left ear only, whereas a value of −1 indicates a response to right-ear stimuli only. Group differences for the individual ROIs were tested by means of two-tailed two-sample *t*-tests.

4.2.7 Network analysis

Pairwise Pearson correlations were calculated as a measure of functional connectivity between all previously defined ROIs. Per group, time courses were determined for all auditory ROIs by averaging the signals of the 10% most active voxels within the ROI and concatenating these averaged signals across the subjects.

Bootstrapping (Wu, 1986; Liu, 1988) was performed to test whether the correlation coefficients between the ROIs were significant within each group separately. In order to obtain a null-distribution for the correlation coefficients, the time courses of random subsets of runs (consisting of 73 points in time) were repeatedly negated for each ROI independently. For each iteration, a Pearson correlation between pairs of ROIs was calculated. A total of 50 000 iterations was performed to produce a null distribution. The obtained null-distribution was used to assess whether significance was reached ($p < 0.05$).

Non-parametric permutation tests (Good, 2002; Nichols and Holmes, 2002) were performed to test whether the correlation coefficients were significantly different between the subjects groups. A null distribution was obtained by randomly reassigning subjects to the two groups, while retaining the original group sizes. A total of 50 000 permutations was performed. For each iteration, the difference between the correlation coefficients was calculated. The obtained null-distributions were used to assess the significance of group difference ($p < 0.05$).

4.3 Results

4.3.1 Subject characteristics

The subjects included in this study had mild to moderate sensorineural hearing loss (see **Figure 4.1**). Statistical analyses of group differences were performed by means of a two-tailed two-sample *t*-test (hearing thresholds and age) or a Fisher's exact test (gender and handedness). Statistical analysis of the hearing threshold at each of the octave frequencies (0.25, 0,5, 1, 2, 4 and 8 kHz, respectively) did not show significant differences between the subjects with and those without tinnitus. The two groups did not differ in age (p = 0.07), gender (p = 0.12), and handedness (p = 1.00). Details of the participants' characteristics are listed in **Table 4.1**.

Age was significantly correlated with hearing loss (regression coefficient m = 0.30 dB/yr; $p = 1.9 \times 10^{-4}$). Out of the 34 tinnitus subjects, 25 subjects perceived tinnitus in both ears, seven subjects perceived tinnitus only in the left ear and two subjects perceived tinnitus only in the right ear. The tinnitus subjects suffered from chronic continuous tinnitus for at least one year up to 29 years.

During the functional scans, the subjects were instructed to press a button whenever they perceived an audible stimulus in the left or right ear. For each stimulus condition, **Table 4.2** lists the percentages of times that the button was pressed by the subjects of the respective group.

4.3.2 Sound-evoked responses

The significant responses to the auditory stimuli presented are visualized in **Figure 4.2**. Based on the *F*-test clear significant responses were detected in the bilateral AC, MGB and IC. Responses in the CN did not reach significance. Additionally, significant responses in the parietal lobe and cerebellum were detected.

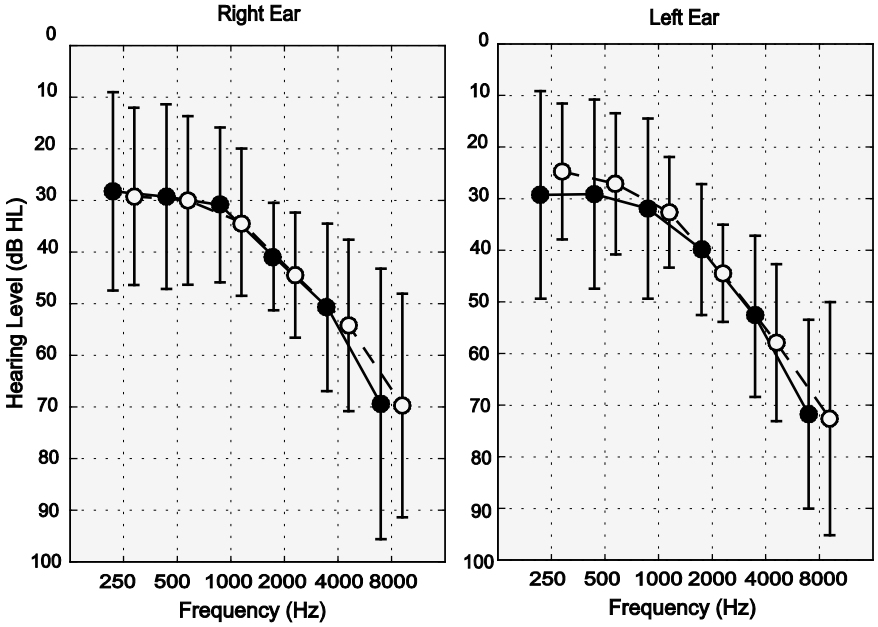

Figure 4.1. Mean audiograms for the HI+T group (solid line) and HI group (dashed line). The error bars indicate the group standard deviations around the mean. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects without tinnitus.

Table 4.1. Subjects' characteristics. Hearing loss was measured as the pure-tone average (PTA) hearing threshold at the octave frequencies 1, 2 and 4 kHz. The mean values with standard deviation are listed. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired; THI: Tinnitus Handicap Inventory.

	HI+T (n = 34)	**HI (n = 19)**
Age		
years	57 ± 10	62 ± 12
range	31 → 75	44 → 84
Gender		
male	21	16
female	13	3
Handedness		
right	28	16
left	2	1
ambidextrous	4	2
Hearing Loss (dB HL)		
right ear	41 ± 8	44 ± 11
left ear	42 ± 10	45 ± 8
THI Score (0 → 100)		
score	31 ± 22	-
range	4 → 72	-

4.3.3 Functional connectivity maps

Pearson correlation coefficients were computed between the time course of each auditory seed ROI and the time courses of all voxels in the brain. The resulting maps, thresholded at an arbitrary level of $R = 0.25$, are presented in **Figure 4.3**. The left and right cortices (AC) are clearly connected to voxels in the thalamus (MGB) and the midbrain (IC), but not with the lower brainstem (CN). The thalamus is connected to voxels in both the cortex and the midbrain and lower brainstem. Correspondingly, the regions in the midbrain and lower brainstem are connected to voxels in the thalamus, but not the cortex.

Remarkably, the auditory cortex and, to a lesser extent, the thalamus show extensive functional connectivity with voxels in the cerebellum, parietal lobe and cingulate cortex. Moreover, the CN shows some connectivity with voxels in the cerebellum. For this reason, these regions were additionally included as two large separate ROIs (i.e. CER and CPR) in the ROI analysis and network analysis that followed.

Table 4.2. Number of times that the button was pressed, expressed in percentages. The percentages are shown for each group and each stimulus condition, respectively. HI+T: hearing-impaired chronic tinnitus patients; HI: hearing level-matched controls without tinnitus.

	HI+T		HI	
	Left ear [%]	**Right ear [%]**	**Left ear [%]**	**Right ear [%]**
30 dB SPL	14	19	16	8
50 dB SPL	52	53	58	52
70 dB SPL	95	94	100	95
90 dB SPL	100	100	100	100

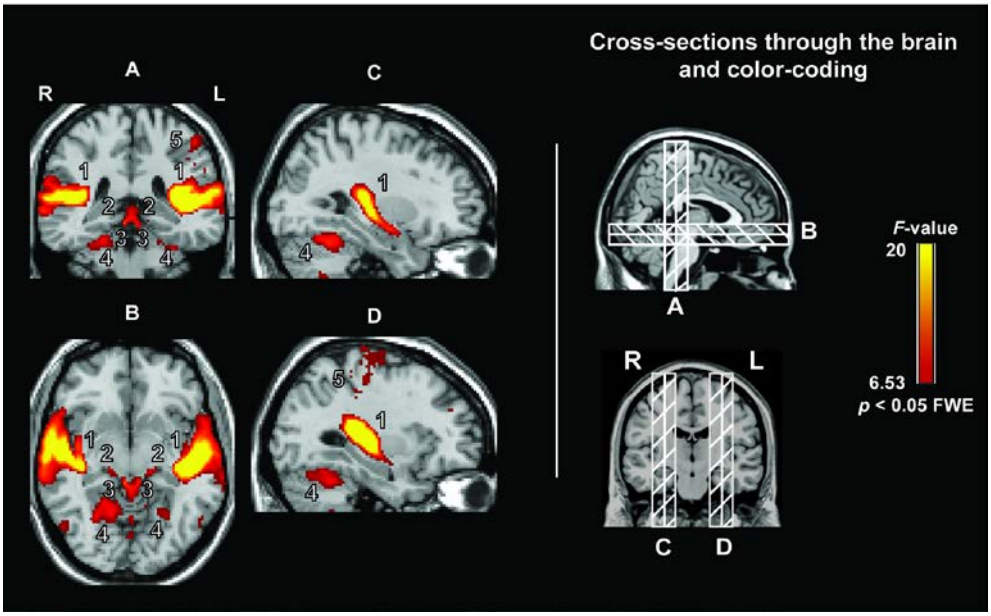

Figure 4.2. Coronal, axial and saggital cross-sections of the brain showing significant responses to sound across all stimulus conditions and all subjects by means of an *F*-test. In each image, the activation in 11 contiguous slices was projected on an anatomical background. The red-yellow color-coded areas indicate areas with a significant response; auditory cortex (1), medial geniculate body (2), inferior colliculus (3), cerebellum (4) and parietal region (5). The threshold was set at $p < 0.05$, corrected for family-wise errors (FWE).

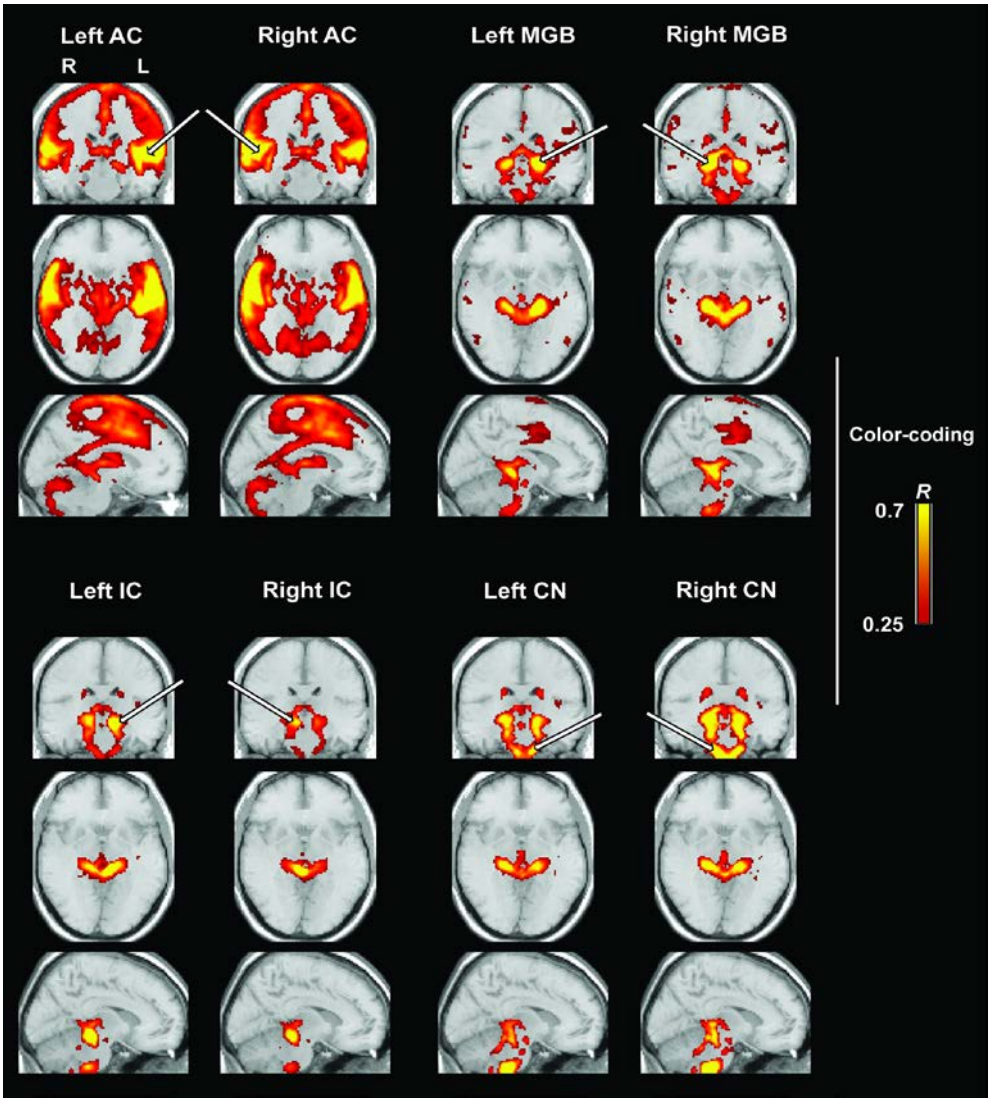

Figure 4.3. Functional connectivity maps across all subjects. Eight auditory regions of interest (ROIs) were defined (indicated with the white arrows). Pearson correlation coefficients *R* were calculated between the time courses of the ROIs and those of every voxel in the brain. The correlation coefficients were thresholded at a level of 0.25, and overlaid on coronal, axial and transversal cross-sections. The red-yellow color-coded areas indicate functionally connected voxels with the respective ROI. Each ROI highly correlated with its constituent voxels and is thus yellow-colored. AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus.

4.3.1 Region-of-Interest analysis

We performed ROI analyses on the eight ROIs in the auditory pathway and the two additional ROIs as defined based on the seed-ROI connectivity maps. For each ROI, the mean percentage signal change for the various stimulus levels compared to baseline in both groups are shown in **Figure 4.4**. In general, louder stimuli yielded larger responses in all ROIs. The CPR and CER showed a plateau effect: responses increased until the stimulus level equaled 70 dB, and subsequently remained at a similar level for the 90-dB stimulus.

For all ROIs, the regression coefficients of the activation as a function of the left and right ear stimulus level were positive (**Table 4.3**). These positive slopes (β values) were significant for all ROIs except the right CN. For hearing loss, a negative regression coefficient was found in all auditory ROIs. Significance was reached in the CER and all auditory subcortical ROIs, except the right IC.

The repeated measures ANCOVA of the ROI responses (**Table 3.3**) showed no effect of subject group (HI vs. HI-T) except in the right CN and left MGB. In all subcortical auditory ROIs, hearing loss had a significant effect on the responses. There was no effect of hearing loss in the auditory cortex. A significant effect of stimulus condition was found in all ROIs except the right CN. In the non-auditory areas (CPR and CER), stimulus condition had a significant effect on the response, but hearing loss only affected the response in the CER.

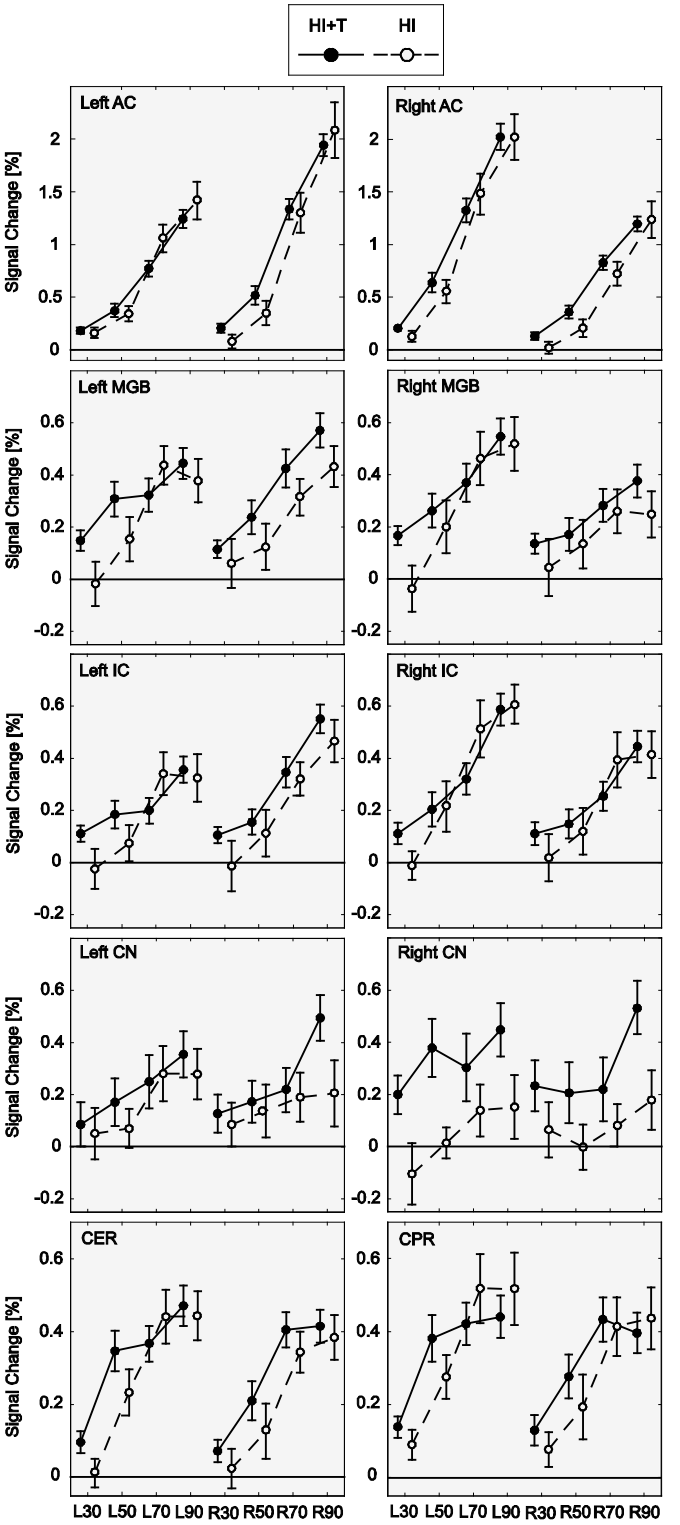

Figure 4.4. The sound-evoked response signals measured in ten ROIs. For each subject group separately, the response averaged across the group is plotted. Hearing-impaired subjects with tinnitus (HI+T): solid lines. Hearing-impaired subjects without tinnitus (HI): dashed lines. The error bars indicate the group standard errors around the mean. AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus; CER: cerebellum; CPR: cinguloparietal region.

Table 4.3. Results of the regression analysis and repeated measures ANCOVA for each ROI. The number of the 10% most strongly responding voxels (N), the regression coefficients (β) of the activation as a function of the left and right ear stimulus level and the hearing loss in the stimulated ear, and the significance (p) of each factor (G: group; S: stimulus; G×S: group-stimulus interaction) or covariate (HL: the subject's PTA hearing loss in the ear of stimulus presentation) according to an ANCOVA are listed. Significant results ($p < 0.05$) are underlined. AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus; CPR: cinguloparietal region; CER: cerebellum.

		Regression coefficient β [10^{-3} %/dB SPL]			Significance p [-]			
	N [2×2×2 mm^3]	Left ear	Right ear	Hearing loss	G	S	G×S	HL
Left AC	499	20	32	-6.3	0.74	<0.001	0.50	0.14
Right AC	453	32	19	-2.1	0.62	<0.001	0.67	0.63
Left MGB	31	5.1	5.9	-7.2	0.021	<0.001	0.36	<0.001
Right MGB	27	7.0	3.5	-6.2	0.08	<0.001	0.45	0.002
Left IC	12	3.4	6.5	-5.4	0.23	<0.001	0.42	0.001
Right IC	15	5.6	3.3	-0.84	0.39	<0.001	0.45	0.62
Left CN	8	4.4	4.4	-7.5	0.15	0.046	0.50	0.004
Right CN	8	3.8	3.7	-13.9	<0.001	0.15	0.59	<0.001
CPR	17402	5.8	5.4	-2.7	0.74	<0.001	0.46	0.15
CER	2241	6.4	6.3	-4.9	0.14	<0.001	0.52	0.003

Figure 4.5 shows the lateralization index for each ROI. The lateralization indices for both groups show a clear contralateral stimulus preference for the bilateral AC, MGB and IC. The right CN was ipsilaterally lateralized in both groups, whereas the left CN was not. The differences between the subject groups did not reach significance. For all nuclei, except the left MGB and bilateral CN, the HI group showed a stronger lateralization towards the left ear, compared to the HI+T group. The CER and CPR tended to lateralize to the left ear.

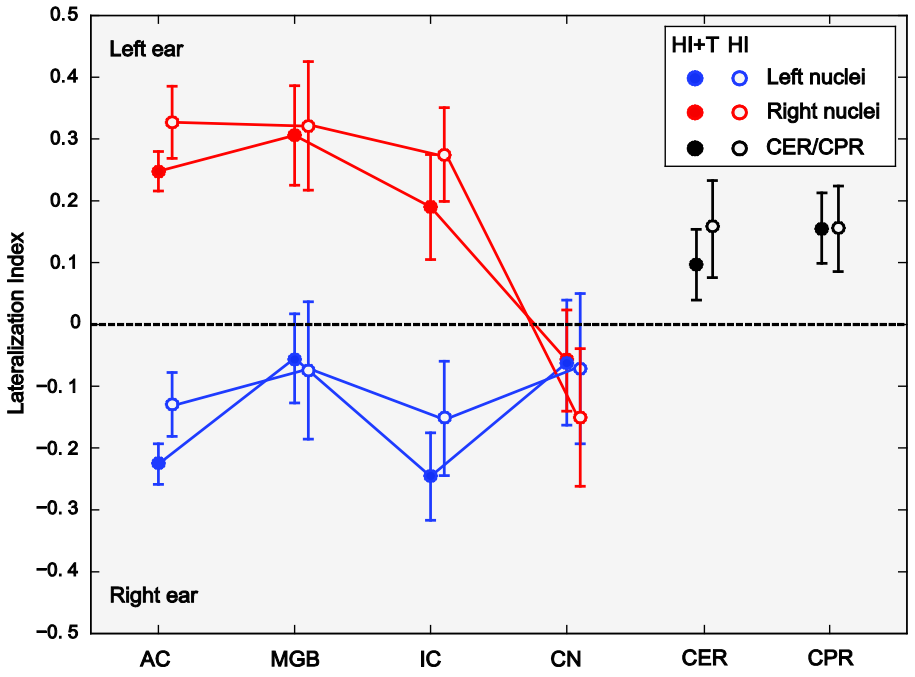

Figure 4.5. The lateralization indices for the left hemispheric auditory ROIs (red) and the right hemispheric auditory ROIs (blue), the cerebellum and the parietal-cingulate region. A lateralization index <0 indicates a response mainly to left-ear stimuli, whereas a lateralization index >0 indicates a response mainly to right-ear stimuli. The error bars indicate the group standard errors around the mean. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects; AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus; CER: cerebellum; CPR: cinguloparietal region.

4.3.5 Network analysis

An overview of the connections for both groups is given in **Figure 4.6**. All possible pairs of ROIs resulted in a significant ($p < 0.001$) positive correlation, where the strongest correlations were found between the contralateral homologues. Successive connections within the auditory pathway were stronger than non-successive connections. The nuclei of the brainstem (CN and IC) were relatively strongly correlated with the thalamus (MGB). The cortex (AC) and the thalamus were strongly correlated as well. However, the correlations between the cortex and

the brainstem were relatively weak. In other words, the correlation analysis showed two clusters of auditory centers, with the thalamus belonging to both clusters. In both groups, the CER as well as the CPR showed the strongest connectivity with the auditory pathway at the level of the cortex. Thus, the CER and the CPR seem to belong to the corticothalamic cluster. Furthermore, the CER and the CPR were mutually highly correlated in both groups (HI+T: $R = 0.72$; HI: $R = 0.74$).

The significant differences between the HI+T and HI groups concerning the functional correlations (indicated by the white squares in **Figure 4.6**) are shown in **Figure 4.7**. The correlations between the (left or right) AC and the (left or right) IC were all significantly higher in the HI group than in the HI+T group. Furthermore, we observed a marginally significant group difference concerning the functional connectivity between the right AC and the CER where the HI+T group again had a lower functional connectivity than the HI group ($p = 0.046$).

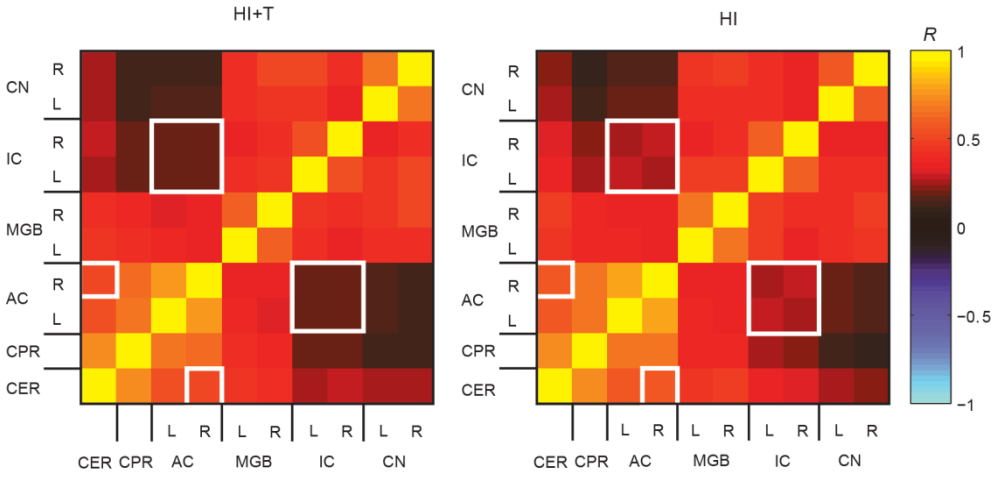

Figure 4.6. Functional connectivity patterns in both the HI+T and the HI group. The color-coding indicates the value of the coefficient of the Pearson correlations (R) between the respective ROIs. Correlation coefficients that show a significant difference between both groups are indicated by a white square. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects; AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus; CER: cerebellum; CPR: cinguloparietal region.

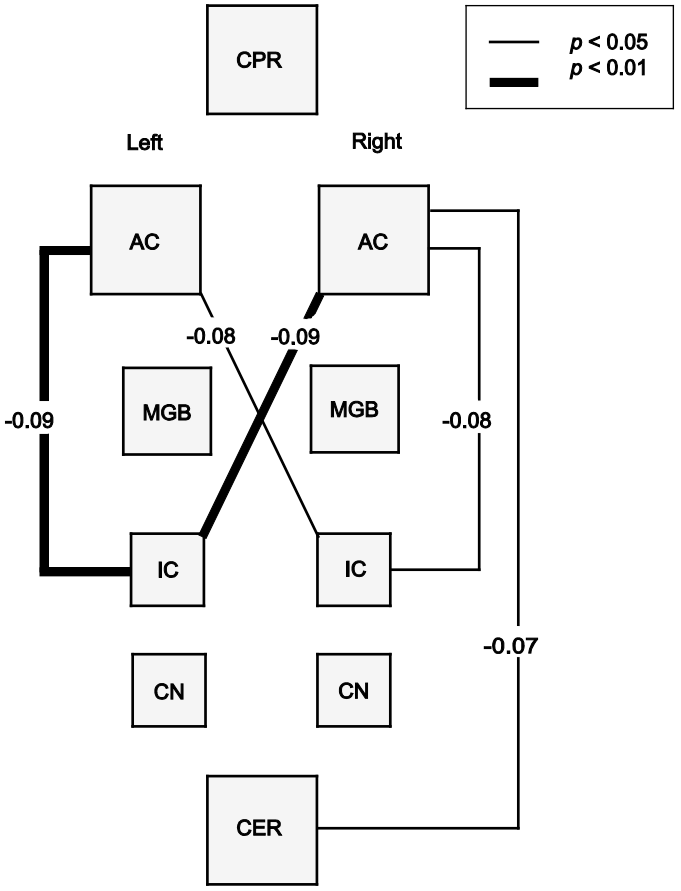

Figure 4.7. Significant differences between the groups for the Pearson correlation coefficients. The numbers along the connecting lines indicate the difference between the correlations of the two groups (tinnitus patients – controls), the thickness indicates the significance of this difference. HI+T: hearing impairment accompanied by tinnitus; HI: hearing-impaired subjects; AC: auditory cortex; MGB: medial geniculate body; IC: inferior colliculus; CN: cochlear nucleus; CER: cerebellum; CPR: cinguloparietal region.

4.4 Discussion

4.4.1 Sound-evoked responses in the central auditory system

Using a 3-T MRI system, activation due to sound stimulation could be detected in multiple regions in the central auditory system of hearing-impaired subjects, including the auditory cortices (AC) in the temporal lobes, the medial geniculate nuclei (MGB) in the thalamus and the inferior colliculi (IC) in the midbrain (**Figure 4.2**). Although our sample of participants was large, no activation could be identified in the lower brainstem (CN) by means of the second-level random-effects analysis. CN activation was only detectable by means of a ROI analysis.

The ROI analysis revealed a clear level-dependency in the cortex, thalamus and midbrain (**Figure 4.4** and **Table 4.3**). The response in each of the auditory brain areas increased with increasing intensity of the sound stimulus, which is in agreement with earlier findings (AC: Hall et al., 2001; Sigalovsky and Melcher, 2006; Langers et al., 2007; Ernst et al., 2008; Lanting et al., 2008; Röhl and Uppenkamp, 2012, Brainstem: Sigalovsky and Melcher, 2006; Lanting et al., 2008; Röhl and Uppenkamp, 2012). In normal-hearing subjects, the relation between brain activation and intensity [expressed in dB] is essentially linear in the auditory pathway (Langers et al., 2007; Röhl and Uppenkamp, 2012). In this study, we found some indications of saturation at loud sound intensity in brainstem and thalamus responses in the hearing-impaired subjects without tinnitus (**Figure 4.4**). Possibly, this difference is related to the reduced dynamic range ('recruitment') that is associated with sensorineural hearing loss.

Activation in the AC, MGB and IC occurred most strongly in response to stimulation of the contralateral ear (**Figure 4.4** and **4.5**). The AC is principally involved in the processing of contralateral stimuli resulting in a distinctly contralateral response upon monaural stimulation (Scheffler et al., 1998; Suzuki et al., 2002; Langers et al., 2005; Langers et al., 2007; Lanting et al., 2008). Correspondingly, for the subcortical brain areas (except the CN), the average responsiveness to the contralateral ear is larger than that to stimulation of the ipsilateral ear (see also, Melcher et al., 2000; Langers et al., 2005; Lanting et al., 2008).

4.4.2 Functional connectivity patterns in the central auditory system

The functional connectivity analysis showed two clusters of auditory brain structures (**Figure 4.6**). Those brain structures are mutually correlated within the respective cluster. The first cluster consists of the brainstem and thalamic nuclei, which showed highly correlated activity patterns. The second cluster contains the thalamic and cortical areas. The activity in these areas showed a highly correlated response as well. Thus, the thalamus is part of both clusters, which is consistent with a function as a relay station between the brainstem and cortex.

This clustering could be related to fundamental differences of the response properties of the various brain areas (for a review, see Eggermont, 2001). One of these differences is related to the temporal properties of the cortical and subcortical responses. While subcortical regions show a sustained response to sound stimuli, the cortex mainly responds to the onset and offset of a stimulus (Harms and Melcher, 2003; Sigalovsky and Melcher, 2006). This means that there is a non-linear relationship between the cortical and subcortical auditory regions. Since we used a linear measure to calculate correlations between the regions, this non-linear relationship might be less well detected.

A further difference in the properties of subcortical and cortical areas, respectively, is reflected by the relation between hearing loss and response amplitudes. While the activation in subcortical areas was significantly diminished for larger hearing losses (**Table 4.3**), such a relation was not present in the auditory cortex. This suggests that the subcortical areas did not adapt to the peripheral hearing loss, while the auditory cortex did adapt, which is consistent with the adaptation that occurs in monaural deafness (Langers et al., 2005).

In tinnitus patients, the correlation coefficient between the cortical and subcortical clusters was diminished relative to the controls (**Figure 4.6** and **4.7**). Since the connection between both clusters is formed by the thalamus, this difference in functional connectivity can be interpreted as a thalamic dysfunction. Interestingly, two models on tinnitus attribute a specific function to the thalamus in the pathophysiology of tinnitus.

The first model (Rauschecker et al., 2010) assumes that the thalamic function is under the control of a subcallosal area. Abnormal gray matter volume and hyperactivity of this area (Mühlau et al., 2006; Leaver et al., 2011) have been hypothesized to lead to abnormal thalamus function causing tinnitus. Our subjects all had a sensorineural hearing loss, which may have caused abnormal neural

activity in the brainstem. This abnormal neural activity is normally blocked by the thalamus. If thalamic gating is impaired, the abnormal brainstem activity may be passed on to the cortex resulting in tinnitus. The abnormal gating in the thalamus of tinnitus patients may also have affected the responses to sound. Specifically, the abnormal connectivity between cortical and subcortical brain areas (**Figure 4.6** and **4.7**) we observed in the tinnitus patients, is possibly related to an abnormality in thalamic gating.

The second model (Llinás et al., 1999) was designed to account for abnormal dysrhythmia observed in the EEG of tinnitus patients. The model assumes that the thalamus in tinnitus patients resides in a hypo-energetic state. This hypo-energetic state is associated with low-frequency bursting activity that is observed in the EEG as an increase of the theta rhythm. It is conceivable that this bursting mode of the thalamus also affects the response of the auditory system to an external sound stimulus. The thalamus may be less accurate to transmit acoustic information from the inferior colliculus to the auditory cortex. Possibly, this accounts for the abnormal connectivity between cortical and subcortical areas, as observed in the present paper (**Figure 4.6** and **4.7**).

4.4.3 The role of non-auditory areas in our experimental paradigm

The CER and CPR were included in our analysis, since their activity correlated extensively with activation in the auditory cortex (**Figure 4.3**). Both regions have been associated with motor control and executive functions (Ghez and Fahn, 1985; Schmahmann and Sherman, 1998; Bush et al., 2000). Obviously, these responses we observed may be related to the button pressing that subjects performed while listening to the experimental stimuli.

Interestingly, the functional connectivity between the AC and CER was lower in the HI+T group as compared to the HI group (**Figure 4.6** and **4.7**). A number of studies has shown an association between tinnitus and cerebellar activity (Brozoski et al., 2007; Osaki et al., 2005; Lanting et al., 2010). Our study contributes another example to these findings, although it remains difficult to interpret the role of the cerebellum in tinnitus.

4.5 Conclusion

We investigated brain responses to sound in subjects with a mild to moderate sensorineural hearing loss, of which a subgroup suffered from tinnitus. The tinnitus and non-tinnitus subject groups were matched with respect to the degree of hearing loss. The most conspicuous differences between the subjects with tinnitus and those without were observed in an analysis of the functional connectivity between brain regions, rather than in the amplitudes of the sound-evoked responses. Specifically, the functional connectivity between the brainstem and cortex was lower in the tinnitus patients. This lower functional connectivity is consistent with tinnitus models proposed by Llinás et al. (1999) and Rauschecker et al. (2010).

ACKNOWLEDGEMENTS

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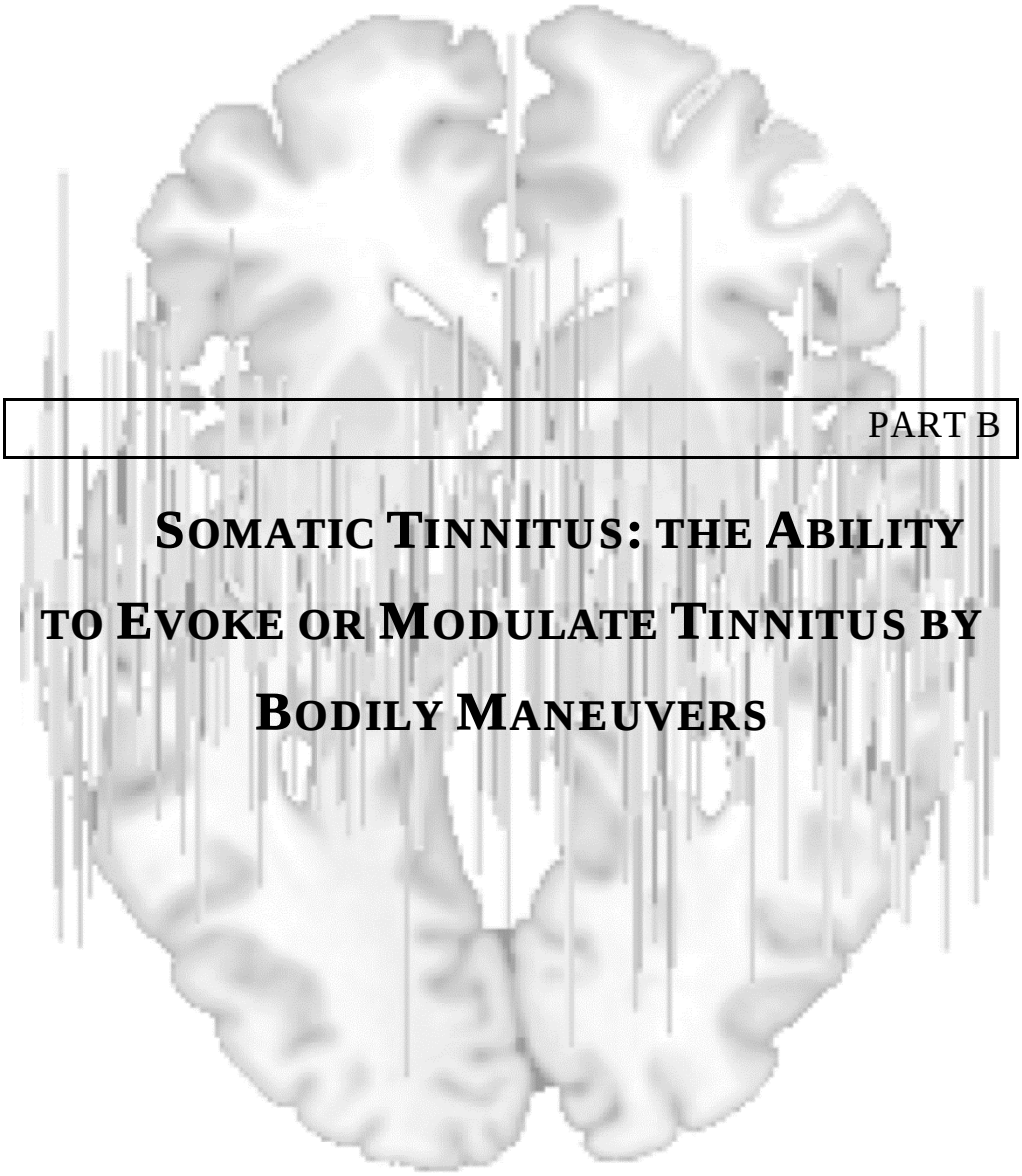

PART B

SOMATIC TINNITUS: THE ABILITY TO EVOKE OR MODULATE TINNITUS BY BODILY MANEUVERS

Chapter 5

Introduction to the Phenomenon of Somatic Tinnitus: Overview of Brain Imaging Studies

5.1 Introduction

Tinnitus is a medical condition in which patients hear sounds, generally described as ringing or hissing in the ears or in the head, which are not audible to others and are not related to an external sound source. Prevalence estimates usually range from 7 to 20% (Hoffman and Reed, 2004). A variety of additional symptoms is often reported, including stress, anxiety, depression, insomnia and irritability (Møller, 2000; Hébert and Lupien, 2007; Langguth et al., 2011). Approximately 40% of the patients with a primary complaint of tinnitus suffer from hyperacusis – an oversensitivity to loud sounds– as well (Baguley, 2003).

Until the present day, the mechanisms underlying tinnitus remain still poorly understood. The generator of tinnitus initially was thought to lie in the inner ear. This hypothesis was put forward because tinnitus is commonly associated with hearing loss (Chung et al., 1984; Levine, 1999a) that usually has a peripheral origin. It is well known that both hearing loss and tinnitus increase with increasing age, which underlines the close correspondence between both phenomena.

If the initial generator of tinnitus lies in the inner ear, it would be expected that dissection of the auditory nerve cures tinnitus. In contrast, dissection of the vestibulocochlear nerve does not eliminate the tinnitus in the majority of subjects (House and Brackmann, 1985; Berliner et al., 1992). Therefore, hypotheses about the underlying pathophysiology have currently been shifted from the periphery to mechanisms in the central auditory system. A generally accepted idea is that tinnitus results from abnormal neural activity in the auditory pathways, which may be triggered by abnormal (or no) peripheral input from the ear to the brain, and is incorrectly interpreted by the brain as a sound (Jastreboff, 1990).

Tinnitus may suddenly start in patients with long-existing peripheral hearing loss. This suggests that some other triggering factors, in combination with the hearing loss, might be responsible for the tinnitus development (Fowler, 1943; Coles, 1996). These triggers may include some classes of medication, psychosocial stress, high-intensity sound exposure, and head and neck somatic factors (Levine, 1999a). Thus, tinnitus may apparently be influenced by non-auditory factors.

A number of clinical observations indicate that many patients are able to voluntarily modulate their tinnitus. Specifically, neck and oro-facial movements, movements of upper extremities, tactile stimulation, eye movements, and teeth manipulations (clenching) have been reported to elicit or modulate the tinnitus (Rubinstein, 1993; Cacace et al., 1994; Pinchoff et al., 1998; Levine, 1999a; Levine,

1999b; Cacace et al., 1999; Sanchez et al., 2002; Levine et al., 2003; Levine et al., 2007; Sanchez et al., 2007; Simmons et al., 2008).

Since 1992, it is known that electrical stimulation of the median nerve can modulate tinnitus loudness in approximately 40% of the patients (Møller et al., 1992). Roughly one-third to 85% of the tinnitus patients report influence on the perceived tinnitus by jaw movements or pressure on the temporomandibular joint (Rubinstein, 1993; Pinchoff et al., 1998). Systematic examination for somatic modulation of tinnitus with head and neck maneuvers reveals a tinnitus modulation in 65 to 80% of the tinnitus patients (Levine, 1999b; Sanchez et al., 2002; Levine et al., 2003; Simmons et al., 2008). Thus, the overall impression from all previous studies is one of a very common occurrence among tinnitus patients.

In this review, we will refer to tinnitus that can be elicited or modulated by somatic maneuvers as somatic tinnitus. Thus, the term somatic tinnitus refers to a range of phenomena; all of which have in common that they involve changes of the perceptual characteristics of tinnitus due to somatic maneuvers.

Having the ability to modulate one's own tinnitus seems to be excellent in order to perform brain imaging studies. The modulatory character of somatic tinnitus offers a unique opportunity to study the relation between tinnitus and brain activity. In this introduction, we explore further the somatic tinnitus phenomenon on the basis of previous brain imaging studies with the purpose of identifying the neural correlates corresponding to the tinnitus modulations.

5.2 Brain imaging studies: identifying the neural correlate of somatic tinnitus

Brain imaging studies were performed in tinnitus patients who could modulate or evoke tinnitus by jaw movements, eye movements and touching the skin. The results of these studies will be subsequently discussed.

5.2.1 Tinnitus modulation by jaw movements

Brain activity patterns associated with tinnitus modulations caused by jaw movements were investigated by Lockwood et al. (1998) and Lanting et al. (2010).

Lockwood et al. (1998) performed a positron emission tomography (PET) study on a small group of four tinnitus patients who could alter the loudness of their tinnitus by jaw clenching. Two of them experienced a loudness increase while jaw clenching (one in the right ear, one in the left ear); whereas the other two subjects reported a loudness decrease (both in the right ear). In addition, a group of six controls without hearing loss or tinnitus was included who were instructed to perform jaw clenching during scanning as well.

In relation to the jaw clenching, the control group showed bilateral activation of the sensorimotor cortex and the supplemental motor area. In the two patients who reported a loudness increase during jaw clenching, increases in cerebral blood flow (CBF) were observed in the sensorimotor cortical regions (similar to the controls), the primary auditory cortex (PAC) and a posterior thalamic region. Group differences between the jaw clenching-induced CBF increases in the controls and those observed in the tinnitus patients were found in the posterior thalamic region including the left medial geniculate body (MGB) in the tinnitus group. The CBF increase in this brain region was interpreted as neural activity that corresponds exclusively to the increases in tinnitus loudness.

There was a difference between those who reported a tinnitus increase and those who reported a tinnitus decrease. In the patients where jaw clenching caused a decrease in the loudness of their tinnitus, a decrease in CBF was found in the posterior and mid-portion of the left middle temporal gyrus. Group differences between the controls and these patients were observed as a reduced CBF in the left temporal lobe and the left hippocampus. The loudness decrease of the tinnitus localized to the right ear was associated with a CBF decrease in the contralateral hemisphere.

Furthermore, an analysis of the data from all three patients with right ear tinnitus revealed a unilateral site of activation in the temporal lobe contralateral to the ear in which they reported their tinnitus: Brodmann areas (BAs) 21 and 41 extending to the hippocampus were the common brain regions where activation was related to tinnitus loudness changes during jaw clenching.

Based on the activation observed in the hippocampus coinciding with the tinnitus loudness, the authors hypothesized that the neural systems mediating tinnitus may be linked to the limbic cortex via the hippocampus.

In a functional magnetic resonance imaging (fMRI) study, Lanting et al. (2010) investigated a group of 13 tinnitus patients that could alter the psychoacoustic characteristics of their tinnitus by jaw protrusion. Among these patients, 77% reported modulations in tinnitus loudness only, due jaw protrusion. Fifteen percent reported a tinnitus pitch change only or the appearance of another sound. One subject experienced both a loudness and a pitch change by jaw protrusion. Loudness changes included mostly a loudness increase. The tinnitus was lateralized to the left in one subject. Another subject reported a lateralization to the right. All other subjects had non-lateralized tinnitus. Additional to the tinnitus group, 20 normal-hearing control subjects without tinnitus were included. Lanting et al. (2010) measured brain responses related to jaw protrusion and sound. In addition, bimodal responses to both jaw protrusion and sound were recorded.

The auditory cortex (AC), MGB, inferior colliculus (IC) and cochlear nucleus (CN) showed a significant response to sound in both subject groups. Jaw protrusion was associated with increased activity in the ventrolateral nucleus of the thalamus, the putamen, the secondary somatosensory cortex and the vermis of the cerebellum. Interestingly, all the mentioned regions of the auditory pathway, except the IC, also responded significantly to jaw protrusion.

The activation in the midbrain and brainstem was different between the tinnitus and the control group. At the levels of the CN and the IC, the tinnitus subjects showed a significantly larger response to jaw protrusion than the control group. The authors argued that the loudness alterations of the tinnitus by jaw protrusion may probably occur due to changes in the normal function of early auditory processing.

The experimental design also allowed to determine which regions respond to a bimodal condition that included somatosensory as well as auditory input. An additional measure of multisensory integration was defined as a significantly larger response to the bimodal condition than to the sum of the unimodal conditions.

Across all subjects, the primary auditory cortex (BA 41) and the auditory association cortex (BA 22) responded significantly to the auditory as well as the somatosensory modality. Evidence for multisensory integration across all subjects was observed in the bilateral middle temporal gyrus (including BA 21), the inferior temporal gyrus, the cingulate gyrus and the somatosensory cortex. In addition, the MGB, the IC and the ventrolateral nucleus of the thalamus show multisensory integration in the control group.

5.2.2 Tinnitus associated with eye movements

Gaze-evoked tinnitus (GET) refers to a phenomenon mainly reported after surgical removal of space occupying tumors from the base of the skull, e.g. a vestibular schwannoma in the cerebellopontine angle (CPA), where tinnitus is modulated or elicited by peripheral gaze. Consequences of this surgery include complete and acute unilateral deafferentation of the auditory periphery and damage to the facial nerve. GET has also been described in cases of acute unilateral deafness, not related to CPA surgery. Since GET may develop without CPA surgery, it has thus been suggested that the acuteness and completeness of the deafness is the triggering factor for the development of GET (Biggs and Ramsden, 2002).

The prevalence of GET after CPA surgery ranges from 19 to 36% (Coad et al., 2001; Biggs and Ramsden, 2002; Baguley et al., 2006). In general, the pre-surgery prevalence of subjective tinnitus (not specifically GET) in patients with a CPA tumor is between 29% and 70%. The post-surgery tinnitus prevalence ranges from 24% to 67% (Andersson et al., 1997; Fahy et al., 2002; Baguley et al., 2005). Furthermore, Andersson et al. (1997) reported a 35-% risk for developing tinnitus when no preoperative tinnitus was present and a 15-% chance that pre-existing tinnitus disappears when present preoperatively.

Three brain imaging studies attempted to identify the neural correlates of GET: Cacace et al. (1995), Giraud et al. (1999) and Lockwood et al. (2001). All subjects described in these papers underwent CPA surgery.

The first imaging study, performed by Cacace et al. (1995), comprised an fMRI examination of one subject in whom the GET was evoked by maintained gaze of the eyes. This subject heard no tinnitus when looking straight ahead. Here, tinnitus-related activity was found in several brain areas in or near the superior colliculus and in the frontal cortex.

Some years later, Giraud et al. (1999) reported the results of a PET study on four subjects who described a loudness change due to eye movements in the horizontal plane (left/right), but not in the vertical plane (up/down). Tinnitus-related activity was calculated by contrasting the images when gazing in the horizontal plane with the images when gazing in the vertical plane. It was shown that phantom auditory sensations enhance activity in the bilateral auditory association areas (BA 21 and 22), but not in the primary auditory cortex (PAC). This gaze-evoked activation is consistently higher in the left hemisphere regardless of the side of the lesion and subsequent tinnitus sensation. The involvement of the auditory association cortices but not the PAC might be explained by pathways that project directly from the MGB to auditory association areas, bypassing the PAC (Møller et al., 1992; Silbersweig and Stern, 1998). Giraud et al. (1999) did not include a control group. Consequently, it is not possible to disentangle the normal brain responses to lateral gaze from those that are specifically associated with tinnitus.

In a second PET study (Lockwood et al., 2001), eight tinnitus patients and seven controls were included. The patients could induce a loudness increase by gazing laterally. Specific GET effects can be entangled by subtracting the responses of the controls from the responses found in the patients. All subjects were instructed to look at a fixation point on the left or right side. Lateral gaze in the GET patients corresponds with increased activity in the auditory brainstem –a region including the vestibular and cochlear nuclei– and auditory cortical sites. Moreover, activation sites are centered in the cuneus and the vermis of the cerebellum. In addition, deactivation in the left temporal gyrus was observed in the control subjects with eye gaze to the left direction.

5.2.3 Cutaneous-evoked tinnitus

A rare form of somatic tinnitus is cutaneous-evoked tinnitus, a condition in which tinnitus is evoked by pressing or stroking a particular area of the body. To our knowledge, only one case study with regard to fMRI has been described (Cacace et al., 1999). In this case, tinnitus was evoked by cutaneous stimulation of the right fingertip regions. The tinnitus was perceived in the deaf ear on the left, where touching the trigger zone elicited a complex auditory percept. Brain activity was found to be predominantly localized to the left hemisphere, contralateral to the cutaneous stimulation. Specifically, tinnitus-related neural activity was detected in the left temporal cortex, i.e. the superior portion of the Sylvian fissure and inferior aspect of the parietal operculum, in the ipsilateral caudate nucleus and in a small

area in the orbital-frontal cortex. Additional activated task-related motor regions were located in the premotor and pre-Rolandic sulcus, and motor areas. When performing a control experiment using the other hand, no activation of the auditory cortex was found, suggesting that the activation found in the auditory region was specifically related to the tinnitus percept.

5.3 Discussion

Somatic modulation of tinnitus is a widespread phenomenon, which may occur due to performing several maneuvers. Most somatic maneuvers trigger a temporary increase of tinnitus loudness instead of a decrease.

5.3.1 Somatosensory-auditory interactions

The occurrence of tinnitus modulation by voluntary movements suggests an abnormal interaction between multiple nervous systems. Levine (1999a) suggested a neurological model of somatic tinnitus, which postulates that tinnitus can arise from somatic-auditory interactions occurring within the central nervous system. In this model, these interactions take place at the level of the CN, which is underpinned by the clinical fact that the tinnitus percept is most often exclusively lateralized to the ipsilateral ear for disorders of the auditory nerve and cochlea.

Recent animal studies showed that somatosensory input originating in the dorsal root ganglia (DRG) and trigeminal ganglion (TG) is ipsilaterally transmitted to the DCN and external nucleus of the IC (Shore et al., 2000; Zhou and Shore, 2004; Shore et al., 2007; Dehmel et al., 2008; Dehmel et al., 2012). Since sensation from the head, neck and face is conveyed by the trigeminal nerve, it is likely that the influence of the jaw and eye movements on the tinnitus is caused by these interactions between the TG and the DCN.

In the event of posterior craniofossa tumor removal, somatic tinnitus in the form of GET has been hypothesized to develop following neuronal sprouting between the auditory system and the system controlling eye movements. The authors of the initial reports hypothesized that GET may be due to cross-modal plasticity, involving axonal sprouting and synapse formation in the central nervous system (Whittaker, 1982; Whittaker, 1983; Wall et al., 1987). Whittaker argued that

regeneration takes place with fibres from the para-abducens nucleus, abducens nucleus or from the median longitudinal fasciculus going to the CN, leading to activation of the auditory pathway. Wall et al. (1987) postulated an abnormal interaction between the vestibular and the cochlear nuclei, also probably subsequent to neural sprouting. As a third possibility, unmasking of a previously inhibited pathway linking eye movement and auditory gain was proposed (Wall et al., 1987; Cacace et al., 1994; Baguley et al., 2006).

Interestingly, most proposed ideas about somatic tinnitus development assigned an important role to the DCN. This would predict that bodily maneuvers result in altered activity in the auditory brainstem. Since the neural activity related to somatic tinnitus is hypothesized to originate in the CN, one may expect that changes in neural activity would be evident throughout the auditory pathway. In agreement with this hypothesis, changed activity related to somatic tinnitus loudness modulation was found in the cochlear nucleus (Lockwood et al., 2001; Lanting et al., 2010), inferior colliculus (Cacace et al., 1995; Lanting et al., 2010), thalamus (Lockwood et al., 1998) and auditory cortex (Lockwood et al., 1998; Cacace et al., 1999; Giraud et al., 1999).

The increased activity in the inferior colliculus and cochlear nucleus is consistent with the models that explain the modulation effect in terms of reorganization of the abducens nucleus or trigeminal projections to auditory brainstem structures. The altered activation further in the auditory system shows that neural activity originated in the CN is transmitted to the successive structures of the auditory system. Therefore, the brain imaging studies performed on somatic tinnitus patients validated the proposed hypothesis.

5.3.2 Possible effects of hearing loss on neural activation

A note that should be made is that the subject characteristics of the groups participating in the described studies were not well matched in terms of peripheral hearing loss. This difference in hearing levels may cause two kinds of effects. Firstly, several studies measured brain responses to external sound stimuli. Then, any difference in brain activity between tinnitus patients and controls may have been caused by the presence of hearing loss, rather than tinnitus. Secondly, even in cases where brain activity was measured in response to a somatic maneuver only, it may still be that the presence of hearing loss affects the responses to a somatic stimulus, despite the fact that no acoustic stimulus was used (Shore et al., 2008). In other words, changes in the central auditory system which follow peripheral

hearing loss make this system reacting differently to other stimuli such as somatosensory input. In none of the imaging studies on somatic tinnitus, the tinnitus and non-tinnitus subjects were matched with respect to hearing loss. Therefore, some caution is appropriate in the interpretation of the currently available results.

5.4 Conclusions

Somatic tinnitus is a common form of tinnitus that offers a unique opportunity to study the relation between brain activity and tinnitus. Although somatic tinnitus is common, some more rare forms of somatic tinnitus (e.g. gaze-evoked and cutaneous-evoked tinnitus) are particularly instrumental in this respect.

Animal studies revealed that the DCN is a principal site of activation between the somatosensory and auditory system (Zhou and Shore, 2004; Shore et al., 2007). If somatic tinnitus is induced by somatosensory input to the DCN, then any tinnitus-related activity would lead to activation throughout the auditory pathway. In the imaging studies, tinnitus-related activity throughout the auditory pathway has been shown, which is consistent with the DCN as the principle interaction site. Future studies on somatic interactions in tinnitus could strengthen the evidence for its role by matching the participating subjects with respect to hearing loss and age.

Chapter 6

Relation between Perception and Brain Activity in Gaze-Evoked Tinnitus

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ABSTRACT

Tinnitus is a phantom sound percept that can be severely disabling. Its pathophysiology is poorly understood, partly due to the inability to objectively measure neural correlates of tinnitus. Gaze-evoked tinnitus (GET) is a rare form of tinnitus that may arise after vestibular schwannoma removal. Subjects typically describe tinnitus in the deaf ear on the side of the surgery that can be modulated by peripheral eye gaze. This phenomenon offers a unique opportunity to study the relation between tinnitus and brain activity. We used functional magnetic resonance imaging to show that in normal-hearing control subjects, peripheral gaze results in inhibition of the auditory cortex, but no detectable response in the medial geniculate body and inferior colliculus. In patients with GET, peripheral gaze (1) reduced the cortical inhibition, (2) inhibited the medial geniculate body, and (3) activated the inferior colliculus. Furthermore, increased tinnitus loudness is represented by increased activity in the cochlear nucleus and inferior colliculus and reduced inhibition in the auditory cortex. The increase of cochlear nucleus and inferior colliculus activity with peripheral gaze is consistent with models of plastic reorganization in the brainstem following vestibular schwannoma removal. The decrease of activity in the medial geniculate body and the reduced inhibition of the auditory cortex support a model that attributes tinnitus to a dysrhythmia of the thalamocortical loop, leading to hypometabolic theta activity in the medial geniculate body. Our data offer the first support of this loop hypothesis of tinnitus, independent of the initial experiments that led to its formulation.

6.1 Introduction

Subjective tinnitus is a common hearing disorder with a potentially devastating impact on the quality of life, characterized by sound perception in the absence of an acoustic stimulus (Lockwood et al., 2002; Langguth et al., 2007; Roberts et al., 2010). It is typically associated with peripheral hearing loss. The physiology of tinnitus may involve increased spontaneous activity, increased bursting activity, or enhanced neural synchrony in the auditory system (Eggermont, 2000; Salvi et al., 2000; Kaltenbach, 2011). Existing studies suggest that tinnitus arises from plastic reorganization that involves a disruption of the normal balance between excitation and inhibition in the brain.

Somatic tinnitus is a special form of tinnitus in which the perceived sound can be elicited or modulated by bodily maneuvers (Levine et al., 2007). Prevalence estimates range from 20 to 78% amongst tinnitus patients (Levine, 1999a; Simmons et al., 2008). One rare example is gaze-evoked tinnitus (GET) in which the tinnitus is modulated or elicited by eye movements. GET has been mainly described after surgical extirpation of a vestibular schwannoma. This surgery leads to severe hearing loss or complete deafness on the surgery side as a consequence of damage to the vestibulocochlear nerve. The prevalence of GET after the surgery ranges from 19 to 36% (Cacace et al., 1994a; Biggs and Ramsden, 2002; Baguley et al., 2006).

Whittaker was the first to report GET (Whittaker, 1982; Whittaker, 1983), hypothesizing that GET may be due to cross-modal plasticity, involving axonal sprouting and synapse formation in the central nervous system. Whittaker argued that regeneration takes place: fibers from the para-abducens nucleus, abducens nucleus or median longitudinal fasciculus invade the cochlear nucleus (CN), leading to activation of the auditory pathway.

The modulatory character of GET offers a unique opportunity to study the relation between tinnitus and brain activity with functional magnetic resonance imaging (fMRI), which depends on functional contrasts between multiple states of brain activation. Moreover, most GET patients have (nearly) normal hearing at the ear contralateral to the surgery, allowing for psychoacoustic evaluation of the tinnitus characteristics by matching external sounds at the hearing ear to the tinnitus heard in the deaf ear (Cacace et al., 1994a; Giraud et al., 1999).

So far, results of three small imaging studies on GET have been published (Cacace et al., 1996; Giraud et al., 1999; Lockwood et al., 2001). These studies showed that tinnitus is accompanied by increased activity in the cerebrum as well

as brainstem, which agrees with hypotheses of hyperactivity in the auditory system (Roberts et al., 2010, Kaltenbach, 2011).

In the present study, psychoacoustic attributes of GET are investigated along with the corresponding neural activity. The spatial resolution of fMRI and the large group of subjects allowed us to identify small changes of activity in the brainstem, thalamus and cortex, and to relate these changes to loudness modulation of the tinnitus percept. Since the neural activity related to GET is hypothesized to originate in the CN, we hypothesized that increased tinnitus loudness would correspond to increased activity throughout the auditory pathway.

6.2 Materials and methods

6.2.1 Subjects

Subjects who perceived GET were recruited through an advertisement on the website of the Dutch society for hearing-impaired patients (NVVS). Eight males and ten females (aged 51±8 years, mean±SD) were included. Of all tinnitus subjects, eight had undergone vestibular schwannoma removal on the left side and ten on the right side. All GET subjects could modulate or evoke their tinnitus by sustained gaze in a direction deviating from the central axis of the eyes. In addition, nine control subjects (five males and four females; 51±10 years) were enrolled. The controls had no tinnitus, hearing complaints or known neurological diseases. None of the subjects reported any psychiatric history. The study was approved by the local medical ethics committee and written informed consent was obtained from each participant.

6.2.2 Psychoacoustics

Standard pure-tone audiometry was performed in the tinnitus subjects as well as the controls. The hearing thresholds were obtained by using an AC440 type audiometer and TDH-39 headphones. The mean audiogram per group is shown in **Figure 6.1**. The control subjects had a mild sensorineural high-frequency hearing loss, despite the absence of any hearing complaints. All tinnitus subjects were interviewed about the characteristics of their gaze-evoked tinnitus by means of a questionnaire. Questions were asked about the time interval between surgery and participation in the study, the presence of pre-existing tinnitus before surgery,

differences between the tinnitus before and the tinnitus after surgery when looking straight ahead, and the first time the subject noticed the GET (**Table 6.1**). Additional information about the diameter of the tumor at the time of surgery, the gradation of the lesion of the facial nerve, and the type of surgery was obtained from clinical files.

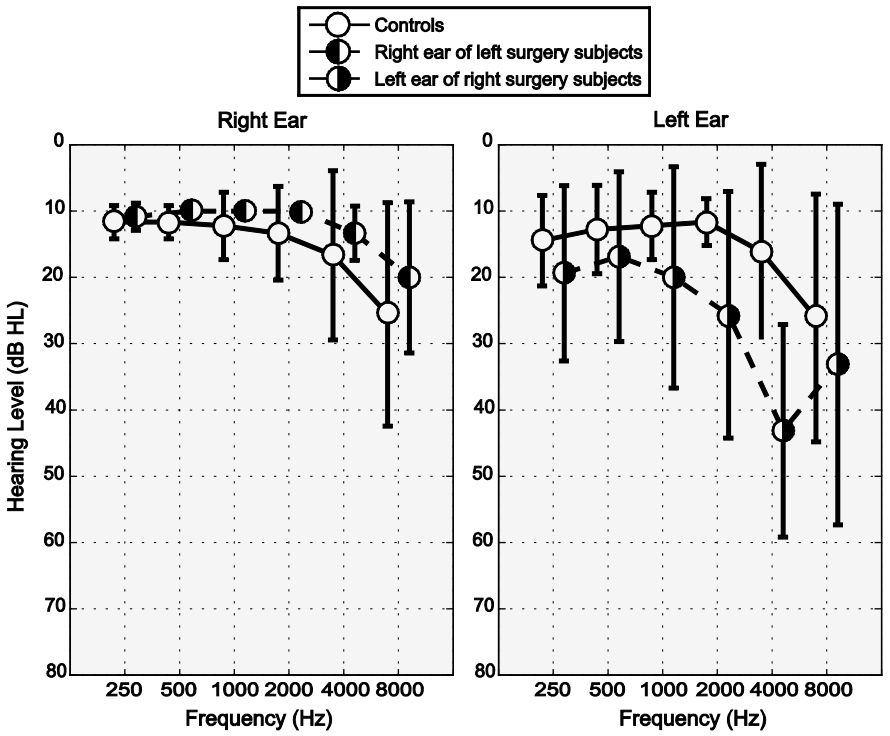

Figure 6.1. Mean audiograms for the controls (n = 9), and the tinnitus subjects who underwent surgery on the left (n = 8) or right side (n = 10). For tinnitus subjects, only the audiograms of the ear contralateral to the surgery are shown. On the side of surgery, the patients were completely deaf, except two, for whom thresholds were between 90 and 120 dB HL. The error bars indicate the standard deviations.

All tinnitus subjects, but not the control subjects, performed a tinnitus matching task for six gaze directions: *Central*, *Left*, *Up*, *Right*, *Down* and *Max*. *Max* was defined as the gaze direction that induced the largest (maximal) subjective change in tinnitus, which could be one of these eight directions: left, upper left, up, upper right, right, lower right, down or lower left (see **Figure 6.2a**). The subjects were instructed to gaze in the indicated direction as far as they could maintain for about ten seconds. The stimuli that were presented in order to match the tinnitus were presented to the unaffected ear opposite to the side of the surgery only.

Initially, the subjects were asked to indicate whether their tinnitus during central gaze most resembled a tone, a narrowband (1/3-octave) noise, or a wideband noise. Next, tinnitus matching was performed for the six gaze directions. If a tone or narrowband noise was chosen to most closely resemble the tinnitus, the procedure was started with a corresponding sound of 1-kHz center frequency, presented at 10 dB above hearing threshold. This sound was adjusted in frequency, employing a step size of 1/8th octave, until subjectively the best matching frequency was reached. Next, the sound level was adjusted in steps of 5 dB until the loudness matched the tinnitus loudness most closely. Starting from the resulting best matching sound, the frequency and loudness matching procedures were subsequently repeated with step sizes of 1/16th octave and 1 dB, respectively. If the subject matched the tinnitus with a wideband noise, only the loudness match was performed. To express the intensity level of the matched sound in dB SL, the hearing threshold for the matched sound was measured during central gaze. The hearing threshold was then subtracted from the intensity level of the matched sound.

6.2.3 MRI data acquisition

All imaging experiments were performed using a 3-T MRI system (Philips Intera, Philips Medical Systems, Best, The Netherlands), which was equipped with an eight-channel phased-array (SENSE) head coil. The functional scans consisted of 2926-ms single-shot T_2*-sensitive echo planar imaging (EPI) sequences with 50 2.5-mm thick slices (TR 10 s, TE 25 ms, in-plane resolution 1.75 mm, field of view 224 × 224 × 125 mm^3). The scans were acquired in ascending order using an oblique transversal orientation, so that the lower part of the pons as well as the upper part of the brain was included. The influence of acoustic scanner noise was reduced using a sparse sampling strategy (Hall et al., 1999; Langers et al., 2005). In our experimental design, there was a 7-s gap of scanner silence between two

successive acquisitions. For each subject, three runs of 76 acquisitions were performed. Additionally, a 3-D high resolution T_1-weighted fast-field echo scan was acquired to serve as anatomical reference.

6.2.4 Scanning paradigm

The paradigm included the same six gaze conditions that were used during the psychoacoustic experiment (*Central*, *Left*, *Up*, *Right*, *Down*, and *Max*). For the *Max* condition, the controls were instructed to look in a direction corresponding to one of the *Max* directions reported by the tinnitus subjects. An additional condition (*Sound*) was included that comprised an acoustic stimulus. The purpose of the condition *Sound* was to be able to delineate the auditory cortex. A bilateral 90-dB SPL dynamic rippled sound (Langers et al. 2003), which was presented while the subjects were looking straight ahead, was never presented while the subjects were performing the gazing task. The spectrum of a dynamic ripple is based on pink noise, but contains spectrotemporal modulations. The stimuli were centered around 1 kHz, with a bandwidth of 5 octaves, a spectral modulation density of 0.1 cycle per octave, a modulation amplitude of 15%, and a temporal modulation frequency of 5 cycles per second, which produces a 5-Hz intensity modulation. Sound was presented by means of MR-compatible electrodynamic headphones (MR Confon GmbH, Magdeburg, Germany; Baumgart et al. 1998) connected to a standard PC with soundcard. Under the headset, subjects wore foam ear plugs to further attenuate the acoustic noise produced by the scanner.

An instruction was delivered to the subject by means of a symbol shown centrally on a projection screen. The symbol consisted of a fixation cross for the *Sound* and *Central* conditions, the text 'MAX' for the *Max* condition, or an arrow in the appropriate direction for the *Left*, *Up*, *Right* and *Down* gaze conditions. The subject performed the task indicated by the symbol during scanning and for the duration of the subsequent silent period. The subject was instructed to look back at the screen when scanner noise was heard, signaling the imminent start of the next trial. In each run, the *Sound*, *Central*, *Left*, *Up*, *Right*, *Down* and *Max* conditions occurred 13, 13, 10, 10, 10, 10, and 10 times, respectively, resulting in a total of 76 trials that were presented in a pseudo-random order.

To monitor whether the task was performed correctly, a 50-Hz MR compatible eye tracker was used, connected via fiber optics to a PC running iViewX software (version 1.0, SMI, Teltow, Germany). A mirror relayed the image of the eye to the infrared camera of the eye tracker, mounted at the foot of the scanner

bed. If the task was not well performed, the corresponding scan was excluded from analysis. Task performance was considered inadequate when the subject did not gaze in the specified direction or excessively moved the eyes during the task. This only happened in six out of 27 subjects, resulting in the exclusion of 15 scans.

6.2.5 Data processing and statistical analysis

The MR images were analyzed using SPM8 (Functional Imaging Laboratory, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/) running under Matlab R2010a (The Mathworks Inc., Natick, MA). The functional images were first corrected for motion using rigid body realignment. During this correction procedure, the orbital regions were masked to minimize the influence of artifacts due to eye movements. Thereafter, the images were spatially co-registered with the T_1-weighted high-resolution anatomical image, and all images were normalized into Montreal Neurological Institute (MNI) stereotaxic space. To improve the signal-to-noise ratio, the functional data were spatially smoothed using an isotropic Gaussian kernel with a full width at half maximum of 8 mm. A logarithmic transformation was carried out in order to express the signal measures in percentage signal change (for details see Langers et al., 2012).

A multiple regression analysis was performed for each subject. The regression model included all seven task conditions, the realignment parameters, as well as a linear term to model drift within each run. Subsequently, the contrast images of the six conditions of interest (*Sound*, *Left*, *Up*, *Right*, *Down* and *Max*), relative to the silent straight ahead condition (*Central*), were fed into a mixed effects group analysis comprising a factorial design with factors for stimulus condition, subject, and group membership (i.e. surgery right, surgery left, and controls).

Because of the co-morbid unilateral deafness, one-sample *t*-tests were used to determine the significant responses to the *Sound* condition for the three subject groups separately. With regard to the five gaze directions, independent samples *t*-tests were performed to calculate the significances of differences between pairs of groups (right surgery, left surgery, and controls). Because no substantial differences were found, the responses to the five gaze conditions were subsequently assessed by performing a one-sample *t*-test on the responses of all subjects. A family-wise error (FWE) corrected confidence threshold of $p < 0.05$ was applied for statistical testing.

6.2.6 Region-of-Interest analysis

In addition to the voxel-by-voxel multiple regression analysis, a region-of-interest analysis was performed. Gaze-evoked responses were assessed in eight anatomical areas comprising parts of the auditory and visual pathways. The cochlear nucleus (CN), inferior colliculi (IC) and superior colliculi (SC) were defined per subject based on their anatomical image. The ROI consisting of the CN included the lower posterior part of the brainstem and was drawn near the cerebellopontine angle (Hawley et al., 2005). The colliculi were drawn on the easily identifiable superior and inferior protrusions of the quadrigeminal plate on the posterior side of the midbrain. The medial geniculate bodies (MGB) and lateral geniculate bodies (LGB) were selected according to the WFU_pickatlas (Maldjian et al., 2003). The bilateral auditory cortex (AC) was defined by means of the regression outcomes: this ROI comprised all voxels in the temporal lobe that were activated by the condition *Sound* in the control group (see Results). Furthermore, a gaze activated area (GAZE+) and a gaze deactivated area (GAZE−) ROI were included. These two areas comprised all voxels that were activated or deactivated by gaze, respectively (see Results).

In order to assess the response lateralization to gaze in all defined ROIs, we also calculated the average activation separately for the left and right regions of interest, respectively. For each group (i.e. left surgery, right surgery and controls), the average activation in each region was calculated for all gaze directions against central fixation.

In each ROI and for both the complete GET group (i.e. left and right surgery combined) and the control group, the average gaze-evoked activation was calculated across the contrast images *Left, Up, Right, Down,* and *Max*. In these ROI analyses, the significance of the response relative to baseline (i.e. central fixation), and the difference between the GET group and control subjects was assessed by two-tailed *t*-tests.

Next, the contrast images of the various gaze directions in the tinnitus subjects were subdivided into three sets according to the loudness increase of the tinnitus perceived by the subject and measured during the tinnitus matching task. This subdivision was performed to determine whether the perceived loudness is represented in the activation of the respective bilateral ROIs. The first set, labeled '≤0 dB', contained all the contrasts for which the subjects did not perceive any loudness increase. This set also contained all contrasts for which decreases in loudness were perceived. A second set labeled '1-5 dB' contained all the contrasts for which the subjects gazed in directions that induced loudness increases up to

and including 5 dB. The third set, labeled '>5 dB', contained all the direction contrasts that caused loudness increases exceeding 5 dB.

Bootstrapping tests (Wu, 1986; Liu, 1988) were performed to test whether the responses in each of the sets significantly differed from zero, respectively. For each set, the random inversion of response amplitudes involved in this procedure was iterated 50 000 times. The 50 000 estimates were used to derive null-distributions in order to assess whether significance was reached ($p < 0.05$).

In addition, non-parametric permutation tests (Good, 2002; Nichols and Holmes, 2002) were performed to test whether significant differences in responses between each set of the GET patients and the controls existed. In order to test the significance of these set differences, the subjects were firstly randomly reassigned to the two subject groups (controls and GET patients), while retaining the original group sizes. Secondly, for the subjects assigned to the GET group, the responses were additionally permuted across the perceptual sets ('≤0 dB', '1-5 dB', '>5 dB'). The resampling of the 135 responses was iterated 50 000 times. For each iteration, the difference between the means was calculated. The 50 000 estimates were combined in order to obtain a null-distribution that was used to assess whether significance was reached ($p < 0.05$).

6.3 Results

6.3.1 Psychoacoustics

The characteristics of the subjects with gaze-evoked tinnitus are listed in **Table 6.1**. The majority of the tinnitus subjects were deaf postoperatively on the side of the surgery; however, in two subjects (# 10 and # 16) hearing remained partially preserved. Removal of the tumor was often accompanied by a paresis of the VII-th cranial nerve. Of all subjects, 12 already perceived tinnitus prior to the tumor surgery. The tinnitus severity had increased in all subjects since the surgery, except in one subject who experienced a decrease and one subject for whom the severity did not change. Ten out of the 18 tinnitus subjects did not remember at which point in time they first noted their tinnitus could be modulated by gaze. The onset-time of the remaining tinnitus subjects ranged from immediately after surgery to 10 months postoperatively.

All tinnitus subjects reported to hear their tinnitus in the ear on the side of the surgery, regardless of gaze direction. Tinnitus modulation due to gaze was similar in a darkened room as compared to a normally lighted room. The effect of gaze was similar when the head was fixed while the eyes changed position, as when the subjects fixated on a cross while turning their head. In other words, the effect on the tinnitus was mainly determined by the orientation of the eyes relative to the skull. In 15 subjects, the effect on tinnitus sustained as long as the gaze remained fixed. Three subjects described that the tinnitus changed only during eye movement, whereas the tinnitus went back to its baseline as soon as the eye position was maintained.

The effect of gaze on the tinnitus characteristics depended on the gaze direction. A systematic evaluation of the effect of gaze while the head was fixed showed a diverse pattern of tinnitus modulations across subjects (**Table 6.2** and **Figure 6.2**). Based on the nature of these modulations, tinnitus subjects were classified in five categories (**Table 6.2**). In three subjects (Category I), gaze resulted in an extra sound, either in addition to a baseline tinnitus that was present when looking straight ahead (# 2 and # 3), or in the absence of such a baseline tinnitus (# 1). The additional sound was perceived on the same side as the original tinnitus. All other subjects described an effect of gaze on the loudness, the pitch and/or the bandwidth of their existing tinnitus. Two subjects experienced a loudness change, while the apparent bandwidth and the pitch remained unchanged (Category II). A tinnitus loudness change was accompanied by a pitch change in six subjects (Category III), and by an additional bandwidth change in five subjects (Category IV). Two subjects described perceiving two distinct tinnitus sounds, which were differently affected by gaze (Category V).

Figures 6.2b-f shows various characteristics regarding the maximally modulated tinnitus percept. Panels b and c are related to gaze characteristics, whereas panels d-f are related to tinnitus characteristics. Despite the diversity of effects, some general trends could be observed: the largest effect on tinnitus loudness was most frequently described for a horizontal gaze direction (panel b), and most often this maximum effect occurred when the subject gazed to the side of the surgery (panel c). Four subjects experienced the largest effect on tinnitus loudness when gazing diagonally: two when gazing up/down ipsilaterally to the side of surgery (# 1 and # 17 in Tables 1 and 2), and two when gazing up/down contralaterally to the side of surgery (# 15 and # 18).

If subjects experienced a change in bandwidth of the tinnitus, it was always a reduction (wideband to narrowband, or narrowband to tonal). Also, the emergence of a new tinnitus sound was always tonal (panel d). Tinnitus loudness

increases occurred more often than decreases (panel e). Tinnitus pitch increases occurred more frequently than decreases (panel f). Pitch changes were always accompanied by a loudness change. Bandwidth changes were always accompanied by both pitch and loudness changes.

Table 6.1. '+': tinnitus arose after surgery; '↓': tinnitus has become less severe; '=': tinnitus remained unchanged; '↑': the tinnitus worsened; '↑↑': the tinnitus worsened a lot; 'I' to 'VI': House-Brackmann grading scale ranging from 'normal' to 'total'; TL: translabyrinthair approach; RS: retrosigmoid approach; '?': not known.

Subject	Surgery side	Months since surgery [years; months]	Tinnitus prior to surgery?	Change of tinnitus severity since surgery	GET first noticed [months since surgery]	Hearing loss ipsilateral [dB HL]	n. VII paresis [House Brackmann]	Tumor diameter [cm]	Surgery type
1	Left	20;8	no	+	<1	>120	VI	?	?
2	Left	22;0	yes	=	?	>120	III	?	RS
3	Right	1;1	yes	↑↑	?	>120	II	1.2	TL
4	Left	19;10	yes	↑	<1	>120	V	4.0	?
5	Right	11;3	no	+	10	>120	II	?	RS
6	Left	0;5	yes	↓	1	>120	II	2.5	RS
7	Right	31;4	no	+	<1	>120	I	?	RS
8	Right	7;7	yes	↑	?	>120	III	3.0	RS
9	Right	13;11	yes	↑↑	?	>120	IV	?	?
10	Left	3;0	yes	↑↑	0	>90	V	?	RS
11	Left	5;10	no	+	?	>120	I	3.0	TL
12	Left	4;4	yes	↑↑	?	>120	?	?	?
13	Right	2;3	yes	↑	>1	>120	I	?	?
14	Right	6;7	no	+	?	>120	I	?	?
15	Left	1;1	yes	↑	?	>120	I	?	?
16	Right	6;9	yes	↑↑	?	>90	VI	2.5	TL
17	Right	6;6	yes	↑↑	?	>120	IV	?	RS
18	Right	10;2	no	+	0	>120	V	?	?

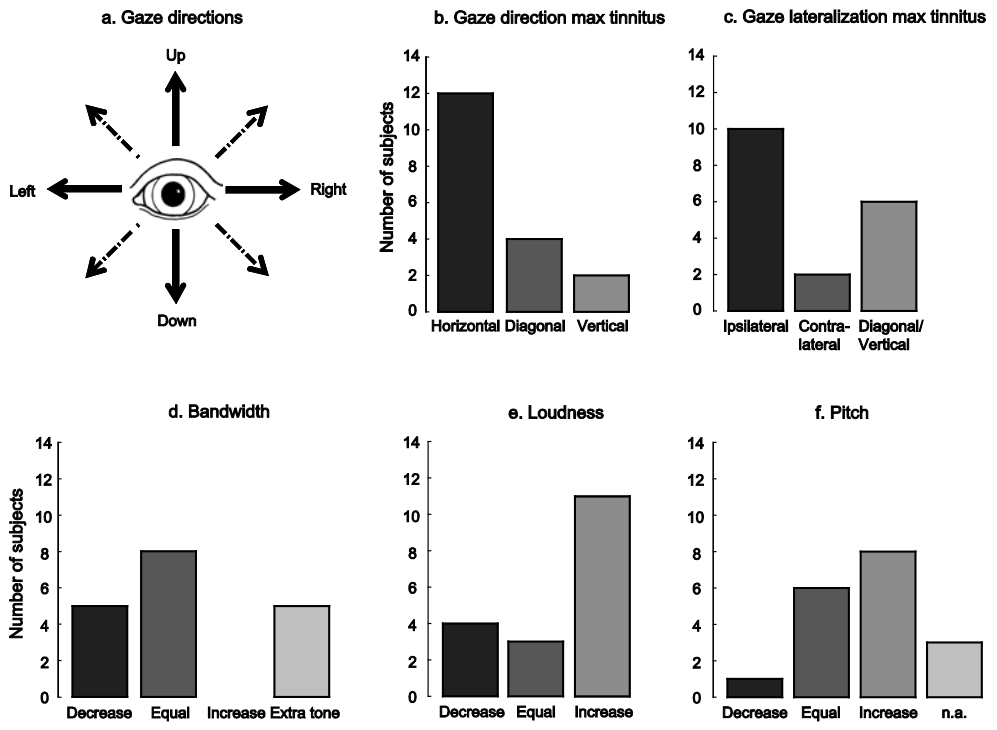

Figure 6.2. The changes to tinnitus were investigated for five gaze directions (panel a): left, right, up, down (solid arrows) and the direction evoking the maximum subjective change of tinnitus (one of the solid or dashed arrows). Histograms showing the characteristics of the perceived tinnitus changes are shown in panels b-f. Panel b shows the gaze direction with largest effect on the tinnitus. Gaze lateralization relative to surgery side with largest effect on the tinnitus is shown in panel c. Changes in respectively bandwidth, loudness and pitch for the maximum tinnitus percept are shown in panels d-f. n.a.: not applicable.

6.3.2 Full brain analysis

The responses to the bilateral sound stimulus in both the tinnitus group and the controls are displayed in **Figure 6.3**. In patients (panels a and b), auditory cortical activation was largest on the side ipsilateral to the surgery. In addition, activation was visible in the IC ipsilateral to the surgery. In the control group, the left and the right auditory cortex showed similar responses to sound, and activation in bilateral

IC and right MGB was observed (panel c). When relaxing the threshold to $p < 0.001$ (uncorrected for multiple comparisons), the left MGB was visible as well.

The brain activation for the different gaze directions, *Left*, *Up*, *Right*, *Down*, and *Max* did not show substantial differences in a comparison between all pairs of subject groups. Therefore, **Figure 6.4** pools the responses of all subjects groups. Horizontal gaze to the left (panel a) yielded activation in the contralateral precuneus and extensive deactivation in the primary visual cortex. Vertical gaze upwards (panel b) resulted in activation dorsally in the occipital lobe and deactivation in the primary visual cortex. Horizontal gaze to the right (panel c) led to activation in the contralateral precuneus and widespread deactivation in the primary visual cortex. Vertical gaze downwards (panel d) and gazing to the *Max* direction (panel e) resulted in some activation mainly coinciding with the inferior areas of the brain regions that were also activated when looking to the left and the right. The dominant effect of downward gaze and gaze to the *Max* direction was extensive deactivation in the primary visual cortex. Panel f shows the *t*-values (i.e. significance) of the averaged activation or deactivation across all subjects and gaze directions.

Table 6.2. *(see next page)* The column headers indicate the gaze directions. Per gaze direction, the bandwidth (B), matched pitch (F, [kHz]) and matched loudness level (L, [dB SL]) are denoted. Bandwidth was either tonal (T), narrowband (NB) or wideband (WB). The column 'Max' indicates the gaze direction ('D') and tinnitus characteristics evoking the maximum tinnitus percept in the subjects. Subjects are grouped with respect to the most prominent effect of gaze on tinnitus. 'I. Extra sound': an (extra) sound arose by eye movements. In subjects #2 and #3, the sound heard when looking straight ahead persisted for all gaze directions. 'II. Loudness change': only the loudness of the tinnitus changed by eye movements. 'III. Loudness and pitch change': both the loudness and the pitch of the tinnitus changed by eye movements. 'IV. Loudness and bandwidth change': both the loudness and the bandwidth of the tinnitus changed by eye movements. In subjects #12 and #16, the pitch of the tinnitus changed as well. 'V. Complex': the changes in the tinnitus induced by gaze corresponded to an alteration in tinnitus loudness. In addition to this alteration, a new tone appeared during performing the eye movements. The direction (D) of the gaze direction that gave a maximum increase of tinnitus loudens is indicated as L: left; LD: left-down; LU: left-up; R: right; RD: right-down; RU: right-up; or U: up. '=': no effect of gaze on the particular tinnitus characteristic; n.a.: not applicable.

Subject	Center			Left			Up			Right			Down			Max			
	B	F	L	B	F	L	B	F	L	B	F	L	B	F	L	D	B	F	L
	I. Extra sound																		
1	-	-	-	T	1.6	20	T	6.5	20	T	1.6	20	T	7	20	LD	T	6.5	22
2	WB	n.a.	13	T	5	5	=	=	=	T	5	5	=	=	=	L	T	5	5
3	WB	n.a.	25	T	9	5	=	=	=	=	=	=	=	=	=	L	T	9	5
	II. Loudness change																		
4	WB	n.a.	18	=	=	20	=	=	=	=	=	19	=	=	=	L	=	=	20
5	NB	4.2	10	=	=	30	=	=	20	=	=	35	=	=	15	R	=	=	35
	III. Loudness and pitch change																		
6	T	8	10	=	9	0	=	10	0	=	9	0	=	=	=	L	=	9	0
7	NB	2	1	=	7.2	4	=	7.2	3	=	7.2	5	=	7.2	2	R	=	7.2	5
8	NB	1.5	15	=	=	=	=	2	12	=	=	=	=	=	=	U	=	2	12
9	T	1.4	19	=	1.5	22	=	1.6	26	=	1.3	22	=	1	22	U	=	1.6	26
10	T	1.7	36	=	2.0	49	=	=	=	=	=	=	=	=	=	L	=	2.0	49
11	T	4.9	17	=	6.3	25	=	=	=	=	6.3	18	=	6	18	L	=	6.3	25
	IV. Loudness and bandwidth change																		
12	WB	n.a.	20	NB	9.5	5	NB	2.5	=	NB	16	16	=	=	=	R	NB	16	16
13	WB	n.a.	0	=	=	=	NB	2.4	4	NB	2.4	10	=	=	=	R	NB	2.4	10
14	WB	n.a.	5	=	=	=	=	=	=	NB	10	15	=	=	=	R	NB	10	15
15	NB	4.4	21	=	=	=	=	=	=	=	=	=	=	=	=	RD	T	1.5	15
16	NB	0.125	10	NB	0.16	15	=	=	=	T	6	25	=	=	=	R	T	6	25
	V. Complex																		
17	NB	9.5	2	=	=	5	=	=	=	=	=	=	=	=	4	RU	=	=	=
	-	-	-	T	9.5	0	=	=	=	T	9.5	3	=	=	=	RU	T	9.5	5
18	NB	0.2	5	=	=	10	=	=	10	=	=	=	=	=	=	LU	=	=	10
	-	-	-	T	2	0	=	=	=	=	=	=	=	=	=	LU	T	2.5	15

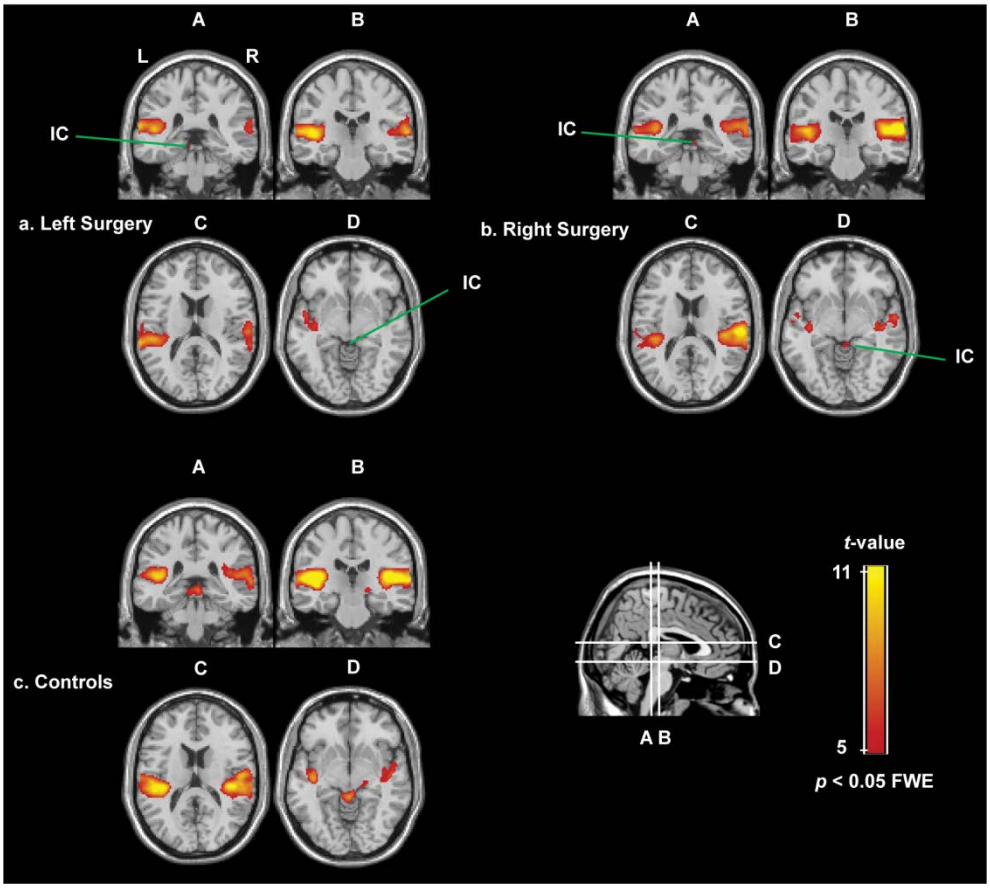

Figure 6.3. Coronal and transversal cross-sections of the brain through the auditory cortex (A-D), medial geniculate body (B), inferior colliculus (A and D) and cochlear nucleus (A) showing significant responses to bilateral 90-dB SPL dynamic rippled sound in a. the subjects who underwent surgery on the left side (n = 8), b. the subjects who underwent surgery on the right side (n = 10), and c. the control subjects (n = 9). The red-yellow color-coded areas indicate areas with a significant response.

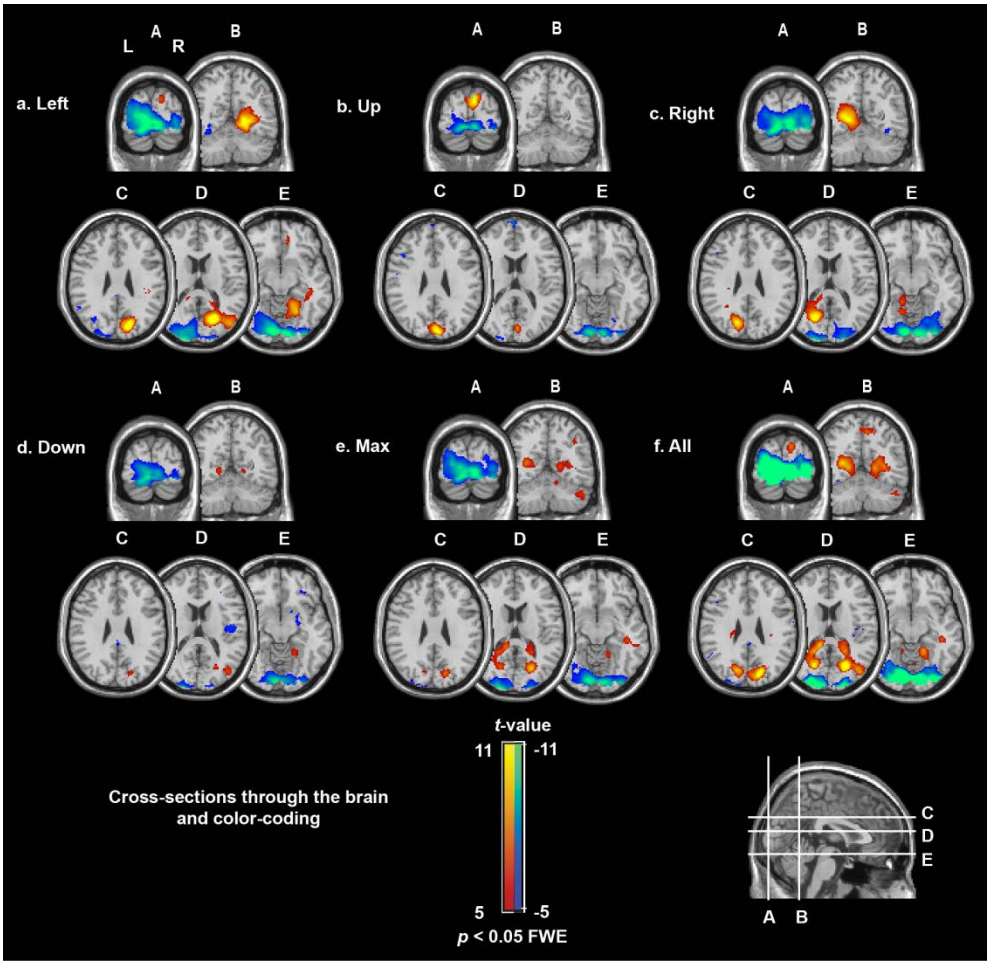

Figure 6.4. Brain responses to the five peripheral gaze directions, contrasted with gazing straight ahead. The responses of tinnitus and control subjects were combined (n = 27). Deactivation was found in primary visual cortex (A-E); activation was found in precuneus (panels a, c, d, e; cross-sections B-E), and dorsally in the occipital lobe (panel b; cross-sections A, C). Panel f shows the average activation and deactivation across all subjects and all gaze directions. The red-yellow color-code indicates areas with a significantly increased activity to gazing; the blue-green color-coded areas indicate areas with a significantly decreased activity.

6.3.3 Region-of-Interest analysis

In addition to the multiple regression analysis, region-of-interest analyses were performed. The ROIs GAZE+ and GAZE− were defined as the occipital areas that responded to peripheral gaze by activation and deactivation, respectively, when averaged across all subjects (**Figure 6.4**, panel f).

Figure 6.5 shows the responses of the cortical and subcortical auditory and visual areas, averaged across all gaze directions. No distinction between hemispheres was made. Peripheral gaze resulted in deactivation of the auditory cortex in control subjects. The CN, the IC and SC, and the MGB and LGB did not show a significant response in the controls. In tinnitus subjects, the auditory cortex showed inhibition but it was significantly reduced in comparison to that in the controls. In contrast to the response in the controls, the IC showed activation, and the MGB and LGB showed deactivation in the tinnitus subjects. Similar to the controls, there was no response in the SC and CN of the tinnitus subjects. The GAZE+ and GAZE− occipital areas were already known to show activation and deactivation, respectively (**Figure 6.4**, panel f), but were not significantly different between patients and controls.

Figure 6.6 shows the lateralization of the activations and deactivations in the regions-of-interest. Here, the three subject groups were considered separately: controls, subjects with left surgery and subjects with right surgery.

One possible result that might be expected in this representation includes different patterns of activation for the left and right surgery subjects. Since the tinnitus is perceived on the side of the surgery, one could predict left-right mirrored activation in both patient groups. **Figure 6.6** shows no evidence of such mirrored activation for both tinnitus groups, respectively.

An alternative possibility would be that a specific hemisphere is involved in the tinnitus, regardless of the side of the surgery. The responses in **Figure 6.6** show some evidence of a specific hemisphere lateralization. In both tinnitus groups, the reduced inhibition in the AC was significant in the left hemisphere, but not in the right hemisphere. Besides, the inhibition of the MGB and LGB was significant in the right hemisphere, but not on the left hemisphere.

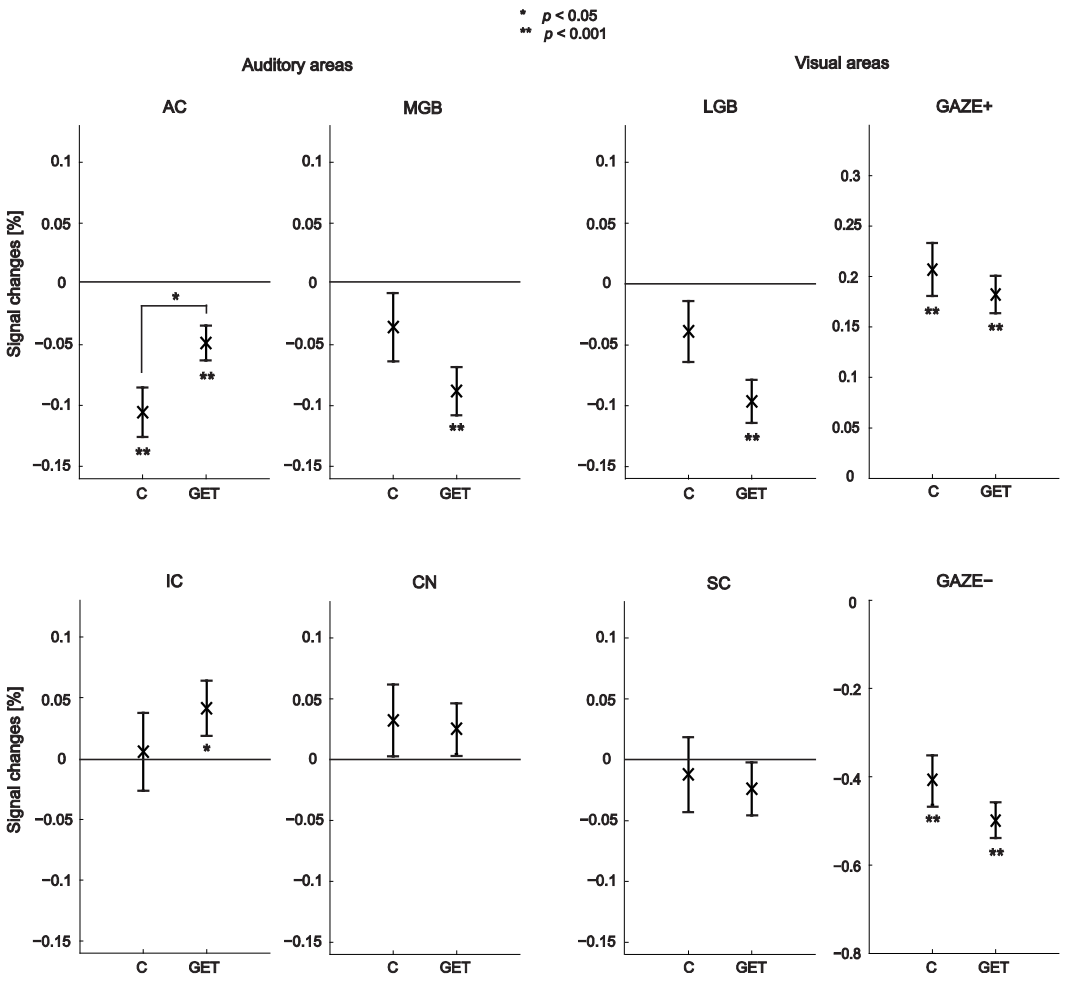

Figure 6.5. Region-of-interest (ROI) responses to peripheral gaze for the controls (C; $n = 9$) and the tinnitus subjects (GET; $n = 18$). Left surgery and right surgery subjects were combined. Also, the left and the right hemisphere, as well as the gaze directions were combined. ROI analyses were performed on bilateral auditory cortex (AC), medial geniculate bodies (MGB), inferior colliculi (IC), cochlear nuclei (CN), lateral geniculate bodies (LGB), superior colliculi (SC), as well as the gaze activated area (GAZE+) and gaze deactivated area (GAZE−). The error bars indicate the group standard errors around the mean. Statistical significances against baseline, and differences between the GET group and the control group are represented by asterisks.

As a next step, the ROI responses corresponding with the various gaze directions in the tinnitus subjects were subdivided into three sets according to the loudness increase of the tinnitus perceived by the subject as measured during the tinnitus matching task, and these results were compared to those from the controls. **Figure 6.7** shows the response levels in the bilateral ROIs per set ('≤0 dB', '1-5 dB' and '>5 dB'). Two subjects (# 15 and # 16) could not hear their tinnitus modulations during scan time. All their responses were included in the '≤0 dB' set. In total, 49, 19 and 22 responses were included in the sets '≤0 dB', '1-5 dB' and '>5 dB' respectively (for a total of 90 responses, corresponding to 18 tinnitus subjects gazing in five directions). Of the 49 responses in set '≤0 dB', 35 correspond to no perceived loudness change and 14 to a loudness decrease. The number of right/left surgery tinnitus subjects for each mentioned set equaled 7/7, 5/2 and 5/3 respectively. The number of right surgery, respectively left surgery subjects exceeds the total number of subjects belonging to both groups, because subjects may have more than one response falling within the various response sets.

The subplots in **Figure 6.7** show various patterns. The bootstrap test showed a significant decrease of the blood-oxygen-level-dependent (BOLD) signal in the AC in the controls and the tinnitus patients. The response levels for the '≤0 dB' and '1-5 dB' sets were significantly decreased, but the significance declines with increasing tinnitus loudness. For the loudest tinnitus ('>5 dB') the signal decrease was not observed. The three GET sets in the MGB showed deactivation, but this was not related to the tinnitus loudness. The deactivations of the '≤0 dB' and '1-5 dB' sets reached significance. Furthermore, significant increases of the IC and CN were detected. The response levels in the sets '1-5 dB' and '>5 dB' of these ROIs showed significant increases compared to baseline. In the LGB, the response levels in the three GET sets showed significant decreases.

Permutation testing revealed a significant difference in means between set '>5 dB' and the controls in the AC. The inhibition in the controls was significant relative to the tinnitus subjects. For the ROI GAZE−, we observed significant decreases for the sets '1-5 dB' and '>5 dB' in the tinnitus group relative to the controls.

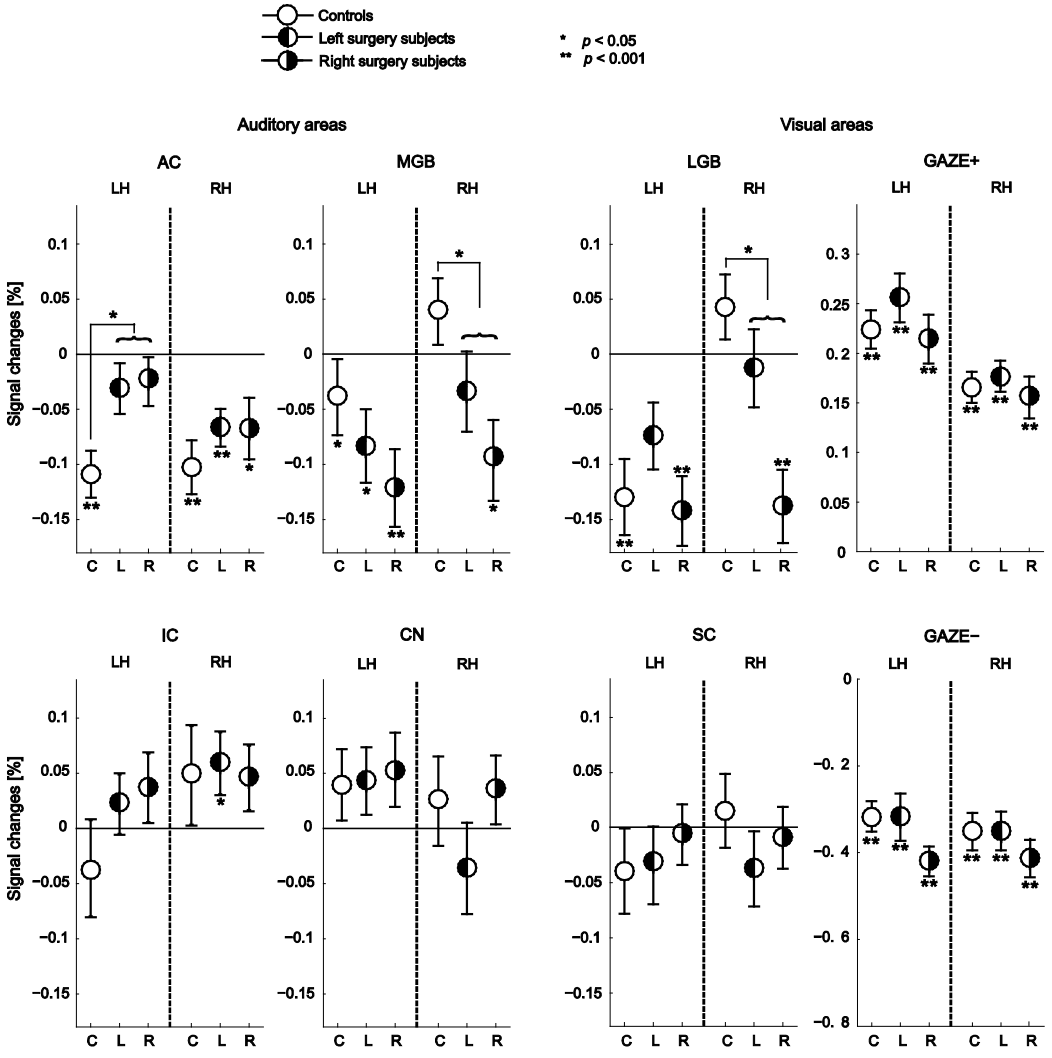

Figure 6.6. Region-of-interest (ROI) responses to peripheral gaze for the controls (C; $n = 9$) and the left surgery (L; $n = 8$) and right surgery (R; $n = 10$) tinnitus groups. In contrast to Figure 6.5, the left and right surgery subjects and the left and the right hemispheres are shown separately. ROI analyses were performed on unilateral auditory cortex (AC), medial geniculate bodies (MGB), inferior colliculi (IC), cochlear nuclei (CN), lateral geniculate bodies (LGB), superior colliculi (SC), as well as the gaze activated area (GAZE+) and gaze deactivated area (GAZE−). The error bars indicate the group standard errors around the mean. Statistical significances against baseline, and differences between the GET group and the control group are represented by asterisks. LH: left hemisphere; RH: right hemisphere.

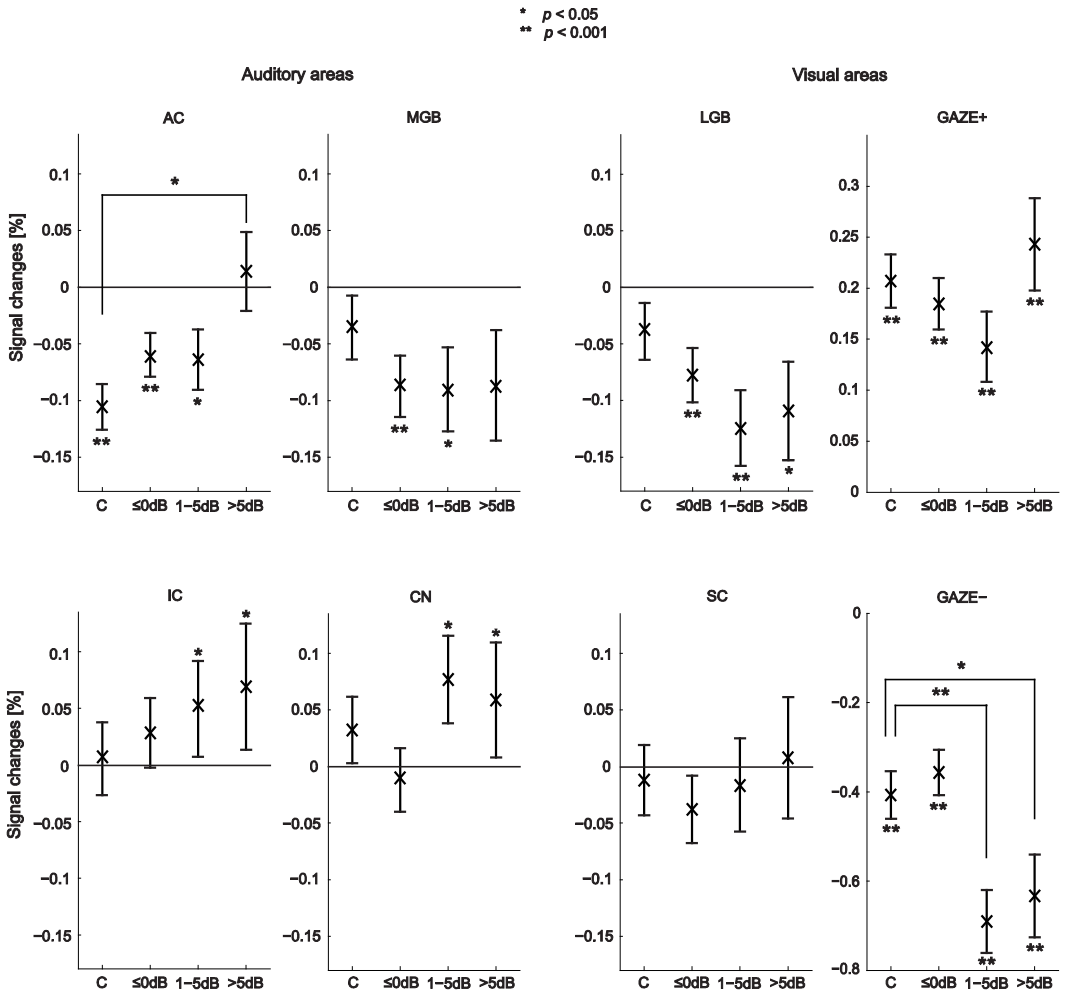

Figure 6.7. Region-of-interest (ROI) responses to peripheral gaze related to change in tinnitus loudness. Controls and tinnitus subjects are shown separately. Control subjects are copied from Figure 6.5. Responses are stratified with respect to the perceived amount of intensity increase of the modulated tinnitus ('≤0 dB', '1-5 dB' and '>5 dB'). In total, 49, 19 and 22 responses were included in the sets '≤0 dB', '1-5 dB' and '>5 dB' respectively. The number of right/left surgery subjects for each mentioned set equaled 7/7, 5/2 and 5/3 subjects respectively. In addition, the ROI responses of the control subjects are shown. The error bars indicate the standard errors around the mean. Statistical significances against baseline, and differences between the sets and the control group are represented by asterisks.

6.4 Discussion

The perceptual characteristics of gaze-evoked tinnitus (GET) and their relation to brain activity were explored. For the gaze maneuvers that changed tinnitus, most often the tinnitus loudness increased, the matched bandwidth decreased or remained unchanged, and the tinnitus pitch usually increased or remained unchanged. An increase in loudness corresponded to an increased BOLD signal in the AC and IC. In tinnitus subjects, peripheral gaze resulted in a deactivation in the MGB; however, the magnitude of the decrease was not related to the tinnitus loudness. To our knowledge this is the first study that shows a relation between tinnitus loudness and simultaneous activity in the midbrain, thalamus, and cortex.

6.4.1 Bandwidth of the tinnitus

The diversity of perceptual characteristics in our tinnitus subjects (**Table 6.2**) is remarkable. Although these subjects had a similar medical history, with removal of a vestibular schwannoma as an important common episode, both the nature of the unmodulated tinnitus and the effect of gaze varied substantially.

Curiously, the stimulus bandwidth that matched the unmodulated tinnitus (column 'center' in **Table 6.2**) varied across our subjects from wideband noise to tonal. In cases of partial high-frequency hearing loss, the tinnitus pitch has been shown to correspond to the frequency range of impaired hearing (Noreña et al., 2002; Sereda et al., 2011), or the edge frequency of the hearing loss (König et al., 2006; Moore et al., 2010). These tinnitus percepts have been interpreted to be the consequence of partial deafferentation: due to the tonotopic organization of the auditory system, loss at certain frequencies is expected to result in tinnitus that is related to the hearing loss frequencies. Our subjects had (nearly) complete loss of auditory function in the surgery ear. Thus, it would be expected that the tinnitus percept covers the entire normal audible frequency range, and would be a broadband percept. The fact that some subjects reported a tonal tinnitus is clearly inconsistent with this expectation. Moreover, if the bandwidth of tinnitus changed due to gaze, it was always a reduction. Apparently, in complete hearing loss, lateral gaze may enhance spontaneous activity or synchronicity in certain frequency channels relative to neighboring channels.

6.4.2 Cortical activity due to gaze

Our fMRI experiment showed that peripheral gaze resulted in both activated and deactivated areas in the occipital lobes (**Figure 6.4**). These effects were observed across all subjects. Deactivated areas coincided with the primary visual cortex. This observation is in line with the results from an fMRI study performed by Andersson et al. (2007). They observed that the activity in the primary visual cortex was modulated by the position of the eyes with a maximum response occurring when both eyes and head orientations were centrally aligned. In other words, deviation from the central axis with the eyes yielded a relative deactivation in the primary visual cortex, which is consistent with our results.

Alternatively, one may explain the deactivation by eye movements to result from a difference in visual input to the eyes. The subjects saw a cross on a screen when looking straight ahead. When the cross was replaced by an arrow, the subject gazed in the direction indicated by the arrow, towards an essentially dark part in the scanner bore. This darkening of the central visual field may account for the deactivation in the primary visual cortex.

In contrast to the deactivation of the primary visual areas, the precuneus activated with peripheral gaze. This brain region is involved in spatial attention (Le et al., 1998; Culham et al., 1998; Simon et al., 2002). The instruction to gaze in various directions may have shifted and enhanced the subject's visuo-spatial attention, resulting in increased activity.

In the control subjects, peripheral gaze caused extensive inhibition of the auditory cortices. This can be interpreted to be a manifestation of cross-modal inhibition, as has been observed in several other studies. For example, engagement in a visual navigation task results in extensive inhibition of the auditory cortices (Drzezga et al., 2005). Similarly, a visual detection task inhibits the auditory cortex (Mozolic et al., 2008). The latter study also showed that processing of visual information is not required per se but that simply directing attention to the visual modality without presenting a target is sufficient to observe the attentional modulation. The present study supports that peripheral gazing alone is sufficient to inhibit auditory areas.

6.4.3 Cross-modal mechanisms and brainstem activity due to gaze

GET typically develops after surgical removal of a vestibular schwannoma in the cerebellopontine angle. In most cases, the surgical removal of the tumor leads to total deafness on the side of the surgery due to transection of the eighth cranial nerve and hence sudden complete deafferentation of the CN in the brainstem (Wiegand and Fickel, 1989; Kane et al., 1995; Bateman et al., 2000). Obviously, the effect of gaze on tinnitus must be an abnormal cross-modal interaction. It has been suggested that deafferentation is a key factor that initiates plastic reorganization, which leads to the abnormal percept (Salvi et al., 2000). The sudden nature of the deafferentation may also account for the reports of GET in other cases of sudden unilateral deafness (Biggs and Ramsden, 2002).

The modulating effect of peripheral gaze on tinnitus has been proposed to originate from reorganization of the functional connections in the lower brainstem. Whittaker (1982, 1983) forwarded that neuronal sprouting from the para-abducens nucleus, abducens nucleus or median longitudinal fasciculus to the CN is responsible for the development of GET. Alternatively, somatosensory input may be involved in the generation of GET. The trigeminal nerve that innervates the face projects to auditory brainstem areas (Shore et al., 2007), which may provide a pathway that mediates the cross-modal interaction. Both these models would predict that peripheral gaze results in increased activity in the auditory brainstem. In line with this prediction, Lockwood et al. (2001) found increased activity of the dorsal CN. In our study, increased activity could be detected in the CN as well as in the IC. Thus, the results of Lockwood et al. (2001) and those presented in the current paper are consistent with models that explain GET on the basis of cross-modal interactions in the brainstem.

6.4.4 Activity across the auditory pathway and tinnitus mechanisms

Our study was initially intended to elucidate the mechanisms of GET specifically. However, from the relations that we observed between the GET loudness and brain activity across the auditory pathway, we may try to draw conclusions on models of tinnitus in general as well.

Notably, we did not observe a relation between the lateralization of tinnitus and that of brain activation. It has repeatedly been shown that monaurally perceived sound results in a response lateralized to the contralateral hemisphere

(Suzuki et al., 2002; Langers et al. 2005; this work, **Figure 6.3**, panels a-b). However, although the tinnitus subjects described their tinnitus to be lateralized towards the deaf ear, a corresponding contralateral lateralization was not present in the brain responses (**Figure 6.6**). Apparently, sounds from external acoustic sources and internally generated tinnitus clearly differ from each other in their representation in the brain.

Arguably the most remarkable finding of our study is that an increase of the tinnitus loudness –accompanied by increased CN and IC activity– did not correspond to an increase of the activity in the MGB. Instead, peripheral gaze decreased MGB activity in the tinnitus subjects in a manner unrelated to loudness. If this decrease is induced by activity in the IC, it must be assumed that the functional connections from the midbrain to the MGB are dominantly inhibitory. This would suggest that the sudden deafferentation due to the tumor surgery reduced the excitatory connections from the IC to the MGB, while the inhibitory connections remained intact.

The decrease of activity in the MGB and the reduced inhibition in the AC has been suggested to be a manifestation of a dysregulation of activity in thalamocortical loops (Llinás et al., 1999). Changes in this loop have been proposed by Llinás and co-workers in order to account for the observation that tinnitus is associated with increased theta and decreased alpha activity of the brain (Llinás et al., 1999; Weisz et al., 2011). They proposed that prolonged hyperpolarization of cells in the thalamus produces low-threshold spike bursts and locks the related thalamocortical loops in low-frequency theta resonance. These low-frequency circuits interact with high-frequency loops at the cortical level, resulting in the generation of a symptom (i.e. tinnitus). The low-frequency hypometabolic areas may be revealed by a reduced blood-supply to the MGB, and hence a decreased BOLD response. In the cortex, the dysregulation of the thalamocortical loop leads to hyperactivity, and hence an increased BOLD response. A decrease in the thalamus and an increase in the cortex has already been found in a study of neuralgic pain (Hsieh et al., 1995). In GET patients, Lockwood et al. (2001) previously reported decreased inhibition in the auditory cortex. Our study is the first to show hypo- and hyperactivity in the thalamus and cortex, respectively, that is associated with tinnitus, as predicted by Llinás et al. (1999).

6.5 Conclusion

Gaze evoked-tinnitus was found to be associated with reduced inhibition of the auditory cortex, increased activity of the cochlear nucleus and inferior colliculus, and inhibition of activity in the medial geniculate body. The increased cochlear nucleus and inferior colliculus activity is consistent with models that explain the modulation effect in terms of reorganization of the abducens nucleus or trigeminal projections to auditory brainstem structures. The inhibition of the medial geniculate body supports a general tinnitus model that explains tinnitus as dysrhythmia of thalamocortical loops, resulting in slow-wave hypometabolic activation of the thalamus.

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Chapter 7

General Discussion and Conclusion

In the studies described in this thesis, tinnitus was investigated by means of functional magnetic resonance imaging and voxel-based morphometry. Two distinct tinnitus patient populations were studied. The first population consisted of tinnitus subjects with mild to moderate hearing loss. The second population consisted of patients who underwent surgical vestibular schwannoma removal and were able to modulate their tinnitus by eye movement. In the following paragraphs, general topics about tinnitus and some methodological aspects will be discussed in relation with the results that were obtained and described in the previous chapters. The next sections contain the opinion of the author of this thesis; therefore, the first person singular forms are used.

7.1 Tinnitus

7.1.1 Evidence for the involvement of more than one central system

Tinnitus is a common complaint characterized by ringing in the ears in the absence of any external sound. Evidence suggests that tinnitus pathophysiology involves damage to the central auditory pathway. However, whether auditory system dysfunction is sufficient to explain chronic tinnitus is unclear, especially in view of evidence concerning distributed networks of auditory and non-auditory cortical and sub-cortical regions. The limbic system is the best known and most commonly reported system involved in tinnitus. Especially with reference to the emotional reaction to the percept of tinnitus, it is not quite surprising that the limbic system, also referred to as the 'emotional brain', plays a role in tinnitus.

The results in this thesis support the idea of the involvement of distributed brain networks in tinnitus. Tinnitus-related functional and anatomical anomalies in auditory as well as non-auditory networks were assessed in several chapters of this thesis. In *chapter 3*, the applied study design allowed a distinction to be made between gray matter effects related to hearing loss and those related to tinnitus alone. It was shown that tinnitus is associated with gray matter increases in the left primary auditory cortex and several areas belonging to the limbic lobe. The tinnitus-related activation changes I reviewed (*chapter 5*) are located throughout the whole auditory system, but also in the hippocampus and cerebellum. In *chapter 4*, I described that in terms of functional connectivity, the strength of the auditory cortex–inferior colliculus and auditory cortex–cerebellum connections are weaker in tinnitus patients.

When I consider all this work together, I conclude that the described results clearly indicate that tinnitus is rooted in a network of brain regions rather than that only functional and structural markers in the auditory system are involved in this phenomenon. This network may be the key to understanding chronic tinnitus.

7.1.2 Tinnitus and its laborious relationship with the thalamus

Both *chapter 4* and *chapter 6* highlight the link between the existence of tinnitus and the functioning of the thalamus. First of all, the weaker functional connection between the auditory cortex and the inferior colliculus may be interpreted as an abnormal gating process of the thalamus in tinnitus patients, since the thalamus acts as a relay between both centers (*chapter 4*). Second, although I reported tinnitus loudness dependency activations in the auditory cortex and the inferior colliculus, this dependency was not observed in the medial geniculate body, which is part of the thalamus (*chapter 6*). Thus, it might be that sudden deafferentation due to tumor surgery reduced the excitatory connections from the inferior colliculus to the medial geniculate body, while the inhibitory connections remained intact.

A specific role of the thalamus in tinnitus is in line with two recent tinnitus models. Rauschecker et al. (2010) proposed an auditory-limbic interaction model in which gating mechanisms in the thalamus are under control of a subcallosal brain region. Deficiency of this control should lead to the onset of chronic tinnitus. Along a different line of thinking, Llinás et al. (1999) suggested that thalamus activity may be in a hypometabolic low-frequency bursting state in chronic and severe neurological or neuropsychiatric disorders including tinnitus. This disturbed state is brought about by excess inhibition of the thalamus by inferior colliculus input, and is supported by the decrease of activity in tinnitus patients reported in *chapter 6* of this thesis.

In my view, these two models serve as an important stepping stone in understanding the generation process of chronic tinnitus. The next step of refinement of these models is necessary to uncover the exact contribution of each of the involved structures to tinnitus. Then, therapeutic targets can be set for e.g. surgical intervention or pharmacological treatment. In the case of Parkinson's disease, for instance, it is known that the excessive inhibition of the thalamus is caused by hyperactive pallidal input onto the motor thalamus. This is assumed because the reduction of thalamic excessive inhibition by electrical deep brain stimulation decreases the pallidal output to the thalamus, which, in turn, diminishes or suppresses Parkinsonian manifestations (Limousin et al., 1998; Fasano et al., 2012). Considering Llinás' model, I suspect that deep brain stimulation may potentially suppress the tinnitus percept. So far, only one study published results about deep brain stimulation in tinnitus patients (Shi et al., 2009), suggesting that deep brain stimulation of the thalamus may indeed provide tinnitus relief for some patients. Remarkably, this relief was caused by electrically stimulating a non-auditory nucleus of the thalamus. Since deep brain stimulation

of the thalamus is technically possibly, I would suggest to explore stimulation of the medial geniculate body in the thalamus as a potential treatment for tinnitus.

7.1.3 What came first, the tinnitus percept or the tinnitus-related brain changes?

Based on my results, it is difficult or even impossible to draw conclusions on the causal relation between the measured brain changes and tinnitus: is tinnitus caused by abnormal structural or functional properties of the brain, or is it the other way around? Below, I will speculate about the causal relation between tinnitus and brain characteristics.

The results in my thesis suggest the involvement of an extensive brain network in tinnitus. This network may be a prerequisite for the conscious perception of sound. In the literature, it has been hypothesized that tinnitus could be generated by abnormal spontaneous neural activity (Eggermont, 2000; Salvi et al., 2000; Kaltenbach, 2011) or by malfunction of a mechanism that normally prevents such activity corresponding with the tinnitus percept (Rauschecker et al., 2010) or both factors. Based on these ideas, I suppose that *functional* changes measured in the auditory system cause tinnitus generation. In my opinion, this causality cannot simply be extended to *structural* changes in the auditory system. The gray matter increase in the left primary auditory cortex of tinnitus subjects may originate as a consequence of ongoing neural activity. Alternatively, this increase could represent a pre-existing vulnerability to develop tinnitus in response to sensorineural hearing loss.

Other parts of the distributed network involved in tinnitus include non-auditory limbic and frontal areas that are respectively associated with emotions and attention. The constant percept of the internal sound frequently causes a considerable amount of distress (Jastreboff et al., 1996) in which both the limbic and frontal regions are involved. Since the distress is due to the tinnitus, I suspect that the changes found in the limbic areas result indirectly from the tinnitus percept. Alternatively, I also believe that the limbic system might trigger the auditory system because many people report that their tinnitus started in a time period of stress. These arguments suggest a bi-directional causal link between changes in the limbic lobe and tinnitus. Furthermore, I also think that potential changes in the limbic and frontal regions related to distress may sustain the chronic tinnitus.

That non-auditory regions may play a more direct role in tinnitus generation is proposed in the tinnitus model of Rauschecker et al. (2010) as well. A subcallosal brain region, which has a pivotal role in that tinnitus model, is an excellent example of direct engagement of a non-auditory region in the emergence of tinnitus.

As a result, the question “what came first?” remains difficult to answer. It seems that both possibilities – first brain changes then tinnitus; first tinnitus then brain changes – are possible. In any case, it is beyond question that understanding the causal relation between the development of tinnitus and the functional and structural brain changes reported in this thesis, will be an important next step in understanding the pathophysiology of tinnitus.

What is needed to understand this causal relation? I suppose that longitudinal studies offer a possible solution. In this kind of studies, subjects are investigated again and again, such that the functional and structural changes of the brain can be followed over time. Since not every hearing-impaired subject does develop tinnitus, the question consequently arises which subjects might be followed. In my view, at least four hearing-impaired subject groups are needed to be enrolled in a probable longitudinal study: subjects without tinnitus who do not have tinnitus sufferers in their families, subjects without tinnitus who have family members suffering from tinnitus, tinnitus patients without other family members suffering from tinnitus, and tinnitus patients with family members suffering from tinnitus. With this study design, the investigator is able to examine brain differences between tinnitus patients and controls without tinnitus, tinnitus-related brain changes in time and, additionally, whether tinnitus is associated with a hereditary vulnerability. Answers on these three issues will give more insight in the pathophysiology of tinnitus.

7.2 Methodological aspects

7.2.1 Differences in results compared to previous studies

Some of the described outcomes appear to contradict or expand the results of previous studies. These differences may be due to a number of causes related to the design of my experiments. Firstly, the relatively large number of subjects participating in the studies presumably led to an increased statistical power (*chapters* 3, *n* = 71; *4*, *n* = 53; and *6*, *n* =27). Secondly, the subject groups were well matched in terms of age and gender (*chapter 6*), and in terms of age, gender, hearing loss and handedness (*chapters 3* and *4*). These factors enabled me to sensitively and specifically look for effects of hearing impairment and/or tinnitus, respectively.

Furthermore, I applied region-of-interest analyses based on relatively large Brodmann Areas (*chapter 3*), group-level outcomes (*chapters 4* and *6*) or anatomical atlases (*chapters 3* and *6*). In the case of the Brodmann Areas, the entire cortex was piecewise explored. The added value of applying these regions-of-interests consists of the possibility to detect weak but spatially extensive changes that may remain undetected in the whole-brain analysis.

My studies on sound-evoked responses were performed by using functional MRI. A major limitation is the acoustic noise produced by the scanner machine. The MRI scanner typically produces over 100 dB SPL of acoustic noise, which makes it difficult to segregate responses to experimental auditory stimuli from those to ambient scanner noise. This problem was tackled by using a sparse imaging design (Hall et al., 1999). In the applied design in my studies, a silence gap of approximately 8 s was inserted between the successive scans, allowing to present experimental stimuli to the subjects during that gap. This ensures that the obtained activation depends on the stimulus alone (Hall et al., 1999).

Together, I believe that the carefully matched and large subject groups may have contributed to an increased sensitivity to alterations of gray matter in the work I described compared to previous studies. In case of the functional MRI studies, the stimulus-related brain activation was enhanced by the additional benefit of the applied sparse design.

7.2.2 Large groups – still small effects – clinical application?

Functional and structural MRI are techniques that enable plastic functional changes and structural differences to be imaged and localized in relative detail. Therefore, MRI seems to serve as a potential method to diagnose tinnitus.

The large number of subjects in our study allowed for the detection of very small tinnitus-related effects. This means that the methods presented here won't be able to detect tinnitus in individual cases in a straightforward manner. However, I showed in *chapter 3* that gray matter analysis of the left primary auditory cortex may detect tinnitus with 80% sensitivity and 70-80% specificity. These probabilities are currently insufficient to serve as practical outcomes in clinical diagnosis and imply that this technique cannot be deployed as a diagnostic measuring instrument yet. But, it is very well possible that the sensitivity and specificity of a VBM analysis may be enhanced by optimization of the detection algorithm.

7.3 Conclusion

This work described tinnitus-related functional and structural differences in hearing-impaired patients. The large number of enrolled subjects, carefully matched subject groups and the application of sparse scanning have contributed to an increased sensitivity to tinnitus specific traits. The presence of tinnitus seems to be associated with an abnormal gating process of the thalamus, which supports two existing tinnitus models, and suggests a potential target for future tinnitus treatments. Furthermore, evidence is given that markers for tinnitus are not only situated in the central auditory system, but that they are embedded in a distributed network consisting of multiple brain regions.

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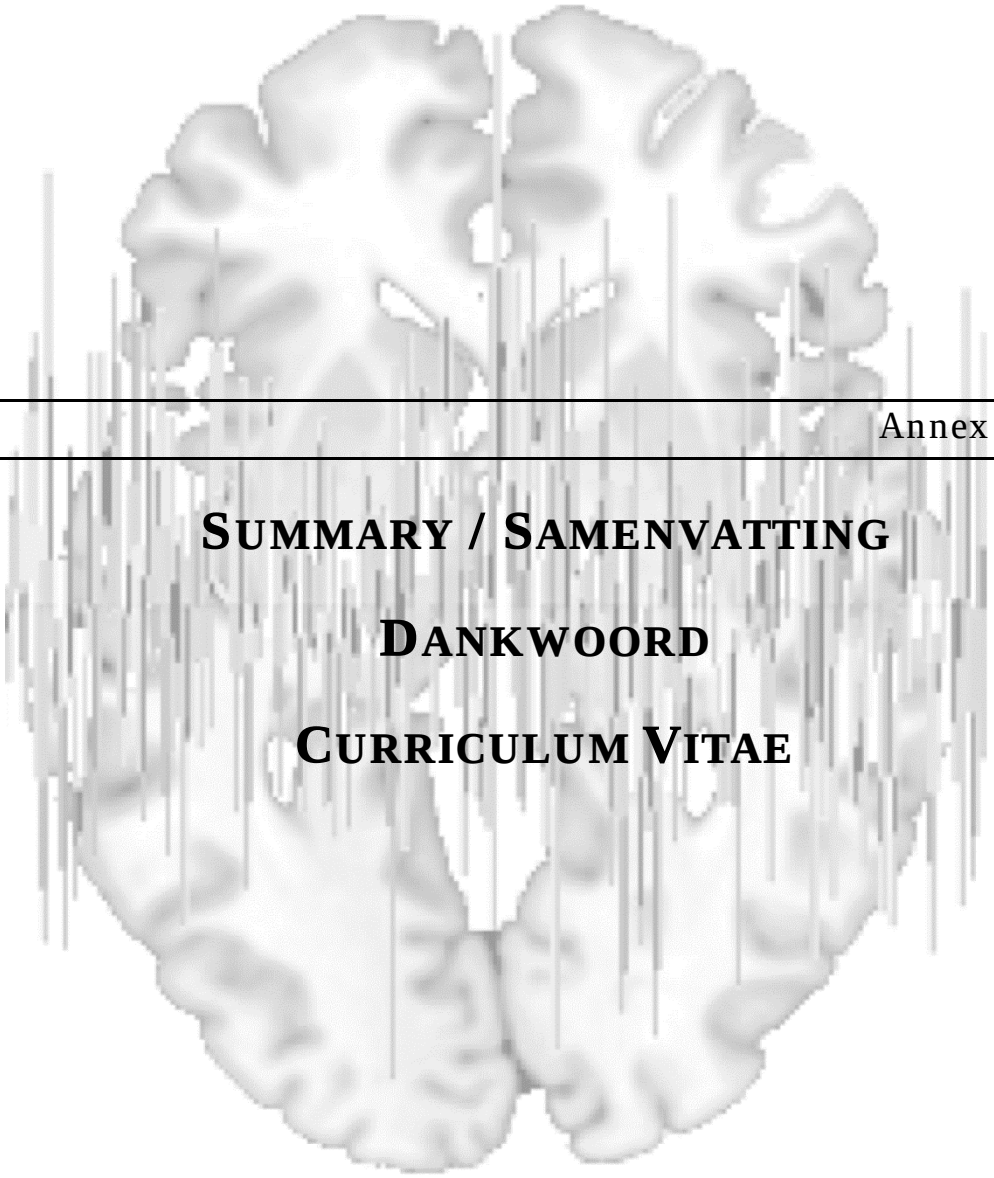

Annex

SUMMARY / SAMENVATTING

DANKWOORD

CURRICULUM VITAE

Summary

Tinnitus, or 'ringing in the ears', is the percept of a sound that is only heard by the patient. Most patients with chronic tinnitus are continuously aware of the tinnitus percept, but are able to cope effectively with the disturbance. However, for some patients the tinnitus is more than a trivial annoyance and has a devastating impact on the ability to function in daily life. These patients often report a variety of additional symptoms including stress, anxiety, depression, insomnia and irritability. Furthermore, tinnitus is often associated with hearing loss.

In this thesis, a number of studies were presented that investigate the human brain in tinnitus patients by means of functional and structural Magnetic Resonance Imaging (MRI). These studies focus on possible brain mechanisms that are involved tinnitus.

Chapter 1 contained a general introduction to tinnitus, the auditory system, hearing impairment and its effects on the central auditory system, and MRI. Additionally, this chapter provided an outline of this thesis.

Part A of this work describes two MRI studies in which a hearing-impaired group of subjects suffering from tinnitus (HI+T group) and a hearing-impaired group of controls (HI group) participated. ***Chapter 2*** gave a detailed overview of the subjects' characteristics who participated in the studies described in ***chapters 3*** and ***4***. In both subject groups, all participants had mild to moderate sensorineural hearing loss, the majority of the subjects were in their fifties or sixties, more males than females were enrolled and the majority of the subjects were right-handed. Consequently, we may conclude that the main difference between both groups is the perception of tinnitus. The perceived tinnitus was subjective, continuous and principally high-frequent in all tinnitus subjects. The tinnitus questionnaires indicated mild to moderate tinnitus in the majority of the subjects. By means of the Hospital Anxiety and Depression Scale, higher scores on both subscales were obtained by the HI+T group than by the HI group. Furthermore, based on the Hyperacusis Questionnaire, the HI+T group suffered more from hyperacusis than the control group.

In ***chapter 3***, the voxel-based morphometry (VBM) approach was used to compare gray matter in the HI+T group and the HI group to a group of controls consisting of normal-hearing subjects. The applied design allowed us to identify the effects that are specific to hearing loss and tinnitus respectively. It was shown that tinnitus is associated with gray matter increases in the left primary auditory cortex

and several areas belonging to the limbic lobe. Overall, we concluded that the most significant effect specific to tinnitus was found in the left primary auditory cortex, where tinnitus is associated with a gray matter increase.

The study described in ***chapter 4*** shows the results of sound-evoked responses and functional connectivity patterns as a probable marker of tinnitus. Across all subjects, the results showed that contralateral stimuli gave larger responses than ipsilaterally presented stimuli in all auditory centers except the cochlear nucleus. Moreover, high-level stimuli gave larger responses than low-level stimuli. Furthermore, significant negative effects of hearing loss on neural activation were demonstrated in the cerebellum and all auditory subcortical regions except the right inferior colliculus. Specifically related to tinnitus, the results did only show an increased sound-evoked response in the left thalamic medial geniculate body and right cochlear nucleus of subjects with tinnitus compared as compared to those without tinnitus. With regard to the functional connectivity patterns, tinnitus subjects showed a significantly decreased correlation between the auditory cortex and inferior colliculus, and between the right auditory cortex and cerebellum as compared to the hearing-impaired subjects without tinnitus.

Part B of this work describes the phenomenon of somatic tinnitus. Many patients are able to modulate their tinnitus by both movements and pressure applied to the head, neck and face. Tinnitus modulations evoked by jaw movements, gaze and skin touching influenced by input different from auditory information suggest to implicate cross-modal plasticity. ***Chapter 5*** contains a review of this occurrence on the base of previous brain imaging studies. Although differences between individual studies, the results of the reviewed studies show a trend that modulated tinnitus corresponds to an increased level of activity throughout the central auditory system. Especially the findings that bodily maneuvers result in altered activity in the auditory brainstem support the thought that the abnormal interactions between the somatosensory and auditory systems take most likely place between the auditory brainstem and the trigeminal ganglion.

In the study defined in ***chapter 6***, a group of subjects who perceived gaze-evoked tinnitus (GET) after surgical removal of a vestibular schwannoma was included. The perceptual characteristics of GET and their relation to activity of the brain were explored. The diversity of perceptual characteristics in our tinnitus subjects is remarkable. Both the nature of the unmodulated tinnitus and the effect of peripheral gaze differed substantially. For the gaze maneuvers that changed tinnitus, most often the tinnitus loudness increased, the matched bandwidth decreased or remained unchanged, and the tinnitus pitch usually increased or remained unchanged. An increase in loudness corresponded to an increase of the

BOLD signal in the auditory cortex and inferior colliculus. The increased colliculus activity is consistent with models that explain the modulation effect in terms of reorganization of the abducens nucleus or trigeminal projections to auditory brainstem structures. Peripheral gaze resulted in a decrease of activity in the medial geniculate body; however, the amplitude of the decrease was not related to the tinnitus loudness. The results are consistent with models that suggest that neurological disorders such as tinnitus are related to an abnormal interaction between the cortex and the thalamus. There are abundant compounds from the thalamus to the cortex and back. Dysregulation of the thalamocortical loop could lead to inhibition of thalamic activity and excitation of the cortex. To our knowledge this is the first study that shows a relation between tinnitus loudness and simultaneous activity in the midbrain, thalamus, and cortex, respectively.

Finally, ***chapter 7*** integrated the results of the various studies, which led to a broad and overarching discussion. The large number of enrolled subjects, carefully matched subject groups and the application of sparse scanning have contributed to an increased sensitivity to tinnitus specific traits. The results of this thesis have shown that tinnitus is rooted in a network of brain regions and not that only the auditory system is involved. Furthermore, the link between the existence of tinnitus and the functioning of the thalamus was highlighted. Both the weaker functional connection between the auditory cortex and the inferior colliculus (*chapter 4*) as the absence of tinnitus loudness dependency activations in the thalamus (*chapter 6*) may be interpreted as an abnormal functioning of the thalamus in tinnitus patients. Finally, we grope in the dark to find an answer to the question *"What came first, the structural/functional changes in the brains or the tinnitus?"*. At this moment, it is not yet possible to demonstrate any causality. Of course, it is beyond question that understanding the causal relation between the development of tinnitus and the functional and structural brain changes reported in this thesis, will be an important next step in understanding the pathophysiology of tinnitus.

Samenvatting

Tinnitus, of een piep in de oren, is bekend als de waarneming van geluid dat alleen door de patiënt zelf wordt gehoord. De meeste patiënten die last hebben van chronische tinnitus zijn zich voortdurend bewust van het tinnitusgeluid, maar kunnen daar goed mee omgaan. Tinnitus kan bij sommige patiënten echter meer veroorzaken dan slechts een klein ongemak waardoor deze stoornis een destructieve invloed heeft op het functioneren van de patiënt in het dagelijkse leven. Deze patiënten vermelden vaak bijkomende symptomen zoals stress, angst, depressie, slapeloosheid en prikkelbaarheid. Tinnitus gaat vrijwel altijd gepaard met gehoorverlies.

In dit proefschrift werd een aantal studies beschreven die de menselijke hersenen van tinnituspatiënten onderzochten middels functionele en structurele *Magnetic Resonance Imaging* (MRI). Magnetic resonance imaging is een techniek die veel voor medische disgnostiek wordt gebruikt om structuren in het lichaam te onderzoeken. Daarnaast is het mogelijk om met functionele MRI activiteit van de hersenen te meten. Het accent lag in dit proefschrift op de mogelijke hersenmechanismes die betrokken zijn bij tinnitus.

Hoofdstuk 1 bevat een algemene inleiding over tinnitus, het auditieve systeem, gehoorverlies en de effecten daarvan op het centrale auditieve systeem, en MRI. Daarnaast beschrijft dit hoofdstuk de opzet van dit proefschrift.

Deel A van dit werk beschrijft twee MRI-studies waaraan een groep slechthorende tinnituspatiënten (HI+T-groep) en een groep slechthorende controles (HI-groep) deelnamen. In ***hoofdstuk 2*** wordt een gedetailleerd overzicht gegeven van de karakteristieken van deze proefpersonen. Beide groepen waren zo samengesteld dat het gemiddelde gehoorverlies en de gemiddelde leeftijd niet verschilden tussen de groepen. Daarnaast waren beide groepen gelijk wat betreft geslacht en handvoorkeur. In beide groepen hadden alle deelnemers een mild tot matig perceptief gehoorverlies, was de meerderheid van de deelnemers een vijftiger of een zestiger, werden meer mannen dan vrouwen geïncludeerd en waren de meesten rechtshandig. Hieruit volgt dat we mogen aannemen dat het waarnemen van de tinnitus het grootste verschil tussen beide groepen vormt. De tinnituspatiënten hoorden allen een subjectieve, continue en voornamelijk hoogfrequente tinnitus. De tinnitusvragenlijsten, die de gevolgen van het oorsuizen meten, gaven aan dat de waargenomen tinnitus een milde tot matige last bezorgde bij de meerderheid van deze patiënten. Twee andere vragenlijsten die vragen naar angst, depressie, en hyperacusis (overgevoloeligheid voor geluid), lieten een

verschil zien tussen beide groepen. Op beide subschalen van de *Hospital Anxiety and Depression Scale* behaalde de HI+T-groep hogere scores dan de HI-groep. Tevens liet de *Hyperacusis Questionnaire* zien dat de HI+T-groep meer last had van hyperacusis dan de HI-groep.

In ***hoofdstuk 3*** werd de "*voxel-based morphometry*"-toepassing gebruikt om grijze massa in het brein te vergelijken tussen de HI+T- en HI-groepen en een controlegroep die bestond uit normaalhorende proefpersonen. Grijze massa heeft als functie het verwerken van informatie. De onderzoeksopzet zorgde ervoor dat we de effecten konden ontrafelen die specifiek gerelateerd zijn aan respectievelijk gehoorverlies en tinnitus. We hebben aangetoond dat tinnitus samengaat met een toename van grijze massa in de linker primair auditieve hersenschors en in verschillende limbische gebieden, ook wel het emotionele brein genoemd. Het meest in het oog springende tinnituseffect werd gevonden in de linker primaire auditieve hersenschors waarbij er een associatie aangetoond kon worden tussen tinnitus en een toegenomen grijze massa aldaar.

De studie die in ***hoofdstuk 4*** beschreven staat, geeft de resultaten weer van responsen op geluid en functionele verbindingen als mogelijke bakens voor tinnitus. Op basis van de resultaten van alle deelnemende proefpersonen zagen we dat contralaterale stimuli een grotere respons lieten zien in alle auditieve gebieden (behalve de *nucleus cochlearis* – onderaan in de hersenstam) dan ipsilateraal aangeboden stimuli. Bovendien resulteerden luide stimuli in grotere responsen dan zachte geluiden. Verder vonden we negatieve effecten van gehoorverlies op de neurale activiteit van alle subcorticale auditieve gebieden behalve de linker *colliculus inferior* (bovenaan in de hersenstam). Specifiek gerelateerd aan tinnitus toonden de resultaten enkel een toegenomen respons op geluid aan in het linker *corpus geniculatum mediale* van de thalamus en de rechter *nucleus cochlearis*. In het brein bestaan vele verbindingen. De analyse van de functionele verbindingspatronen geeft kwantificatie van de sterkte van deze verbindingen. Met betrekking tot de verbindingspatronen werd er bij de tinnituspatiënten een verminderde verbindingssterkte tussen de auditieve hersenschors en de *colliculus inferior*, en de auditieve hersenschors en het *cerebellum* (de kleine hersenen) aangetoond.

Deel B van dit werk beschrijft het fenomeen somatische tinnitus. Veel patiënten kunnen hun tinnitus moduleren, bijvoorbeeld door bewegingen te maken met de kaak, of door druk uit te oefenen op het hoofd, de nek en het aangezicht. Deze tinnitusmodulaties die ontstaan door andere input dan auditieve informatie doen vermoeden dat er sprake is van cross-modale plasticiteit. ***Hoofdstuk 5*** geeft een overzicht van eerder uitgevoerde beeldvormingsstudies bij patiënten met

somatische tinnitus. Hoewel er een grote variatie bestaat tussen deze studies, geven de resultaten toch aan dat er sprake is van een trend, namelijk dat tinnitusmodulatie samengaat met toegenomen activiteit in het gehele auditieve systeem. Vooral de vaststelling dat lichaamsbewegingen veranderingen in de activiteit van de hersenstam veroorzaken, onderbouwt het vermoeden dat de abnormale interacties tussen het somatosensorische en auditieve systeem het meest waarschijnlijk plaatsvindt tussen de auditieve hersenstam en het *ganglion trigeminale* (zenuwknoop van een gevoelszenuw van het aangezicht).

In de studie beschreven in ***hoofdstuk 6*** nam een onderzoeksgroep deel waarvan de proefpersonen tinnitusmodulaties waarnamen door oogbewegingen te maken (GET – *gaze-evoked tinnitus*). Al deze patiënten hadden een chirurgische ingreep ondergaan waarbij een brughoektumor verwijderd werd. We onderzochten de perceptuele karakteristieken van de GET en hun relatie met hersenactiviteit. Zowel de aard van de niet-gemoduleerde tinnitus als het effect van de perifere oogbewegingen verschilden aanzienlijk tussen deze tinnituspatiënten. Meestal veroorzaakten de oogbewegingen een luidheidstoename van de tinnitus, een afname van de bandbreedte of geen verandering qua bandbreedte, en een toegenomen of onveranderde toonhoogte van de tinnitus. De toegenomen tinnitusluidheid kwam overeen met een toegenomen activiteit in de auditieve hersenschors, de *colliculus inferior* en de *nucleus cochlearis*. Deze toename in de laatste twee structuren is consistent met modellen die het modulatie-effect uitleggen in termen van reorganisatie. Zenuwvezels van de *nucleus abducens* (zenuwknoop van een oogzenuw die de oogspieren innerveert) of het *ganglion trigeminale* projecteren naar de auditieve structuren gelegen in de hersenstam. De oogbewegingen veroorzaakten een activiteitsafname in het *corpus geniculatum mediale*, maar deze afname was niet gerelateerd aan de luidheid van de tinnitus. Deze afname en de toegenomen activiteit in de auditieve hersenschors is mogelijk een indicatie van een ontregeling van activiteit in de thalamocorticale loops. Voor zover wij weten is dit de eerste studie die een relatie aantoont tussen tinnitusluidheid en simultane activiteit in respectievelijk de *nucleus cochlearis*, de *colliculus inferior*, het *corpus geniculatum mediale* en de auditieve hersenschors.

Hoofdstuk 7 integreert ten slotte de resultaten uit de diverse studies wat uitmondt in een brede en overkoepelende discussie. Het grote aantal deelgenomen proefpersonen en de zorgvuldige samenstelling van de groepen, maar ook de toegepaste scantechniek hebben geleid tot een grote gevoeligheid om tinnitusspecifieke eigenschappen op te sporen. Uit de resultaten van dit proefschrift is naar voren gekomen dat tinnitus geworteld is in een netwerk van hersengebieden en niet dat alleen het auditieve systeem erbij betrokken is. Daarnaast werd de link gelegd tussen het hebben van tinnitus en het functioneren

van de thalamus. Zowel de zwakkere verbinding tussen de auditieve hersenschors en de *colliculus inferior* (*hoofdstuk 4*) als het niet kunnen vinden van luidheidsafhankelijke activatie in de thalamus (*hoofdstuk 6*) kan geïnterpreteerd worden als het abnormaal functioneren van de thalamus bij tinnituspatiënten. Tot slot tasten we nog in het duister wat betreft het formuleren van een antwoord op de vraag "*Wat kwam eerst, de structurele/functionele veranderingen in de hersenen of de tinnitus*?". Op dit moment is het nog niet mogelijk om enige causaliteit aan te tonen. Wel staat buiten kijf dat het ontdekken van deze causale relatie een belangrijke volgende stap vormt voor het kunnen begrijpen van het fenomeen tinnitus.

Dankwoord

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Curriculum Vitae (English)

Kris Boyen was born in Jette (Brussels, Belgium) on June 21, 1986. She followed pre-university education at the Heilige Drievuldigheidscollege in Leuven. Thereafter, she went to the Catholic University of Leuven to study “Speech Therapy and Audiology”. During her masters, she specialized in “Audiological sciences”. For her thesis, she studied the relationship between auditory steady-state responses to stimuli presented above the threshold of hearing and speech understanding. This study was performed in normal-hearing as well as hearing-impaired adults. She went to the Ear Nose Throat (ENT) department of the University Medical Center Groningen (UMCG) for her clinical and scientific training. There, she did research on the perception of pitch differences by patients with a cochlear implant.

After earning her master's degree in 2008, she started a PhD at the ENT department of the UMCG. The doctoral research dealt with responses in the brain in tinnitus patients that were measured by using functional Magnetic Resonance Imaging (MRI). In addition, she studied structural brain changes associated with hearing loss and tinnitus. This research led to the defense of her thesis and obtaining the doctoral degree in 2013.

Subsequently, she started as a postdoctoral researcher at the ENT department of the UMCG, where she studies gap detection in tinnitus patients. She also accepted a teaching position at the University of Applied Sciences (‘Hanzehogeschool”) in Groningen. There, she teaches the courses acoustics and audiology, and provides scientific support to the program "Speech Therapy". Furthermore, she works as a guest lecturer audiology in the "Speech Therapy" program offered by Windesheim Flevoland in Almere.

Curriculum Vitae (Nederlands)

Kris Boyen werd op 21 juni 1986 geboren te Jette (België). Ze doorliep haar middelbare schooltijd op het Heilige Drievuldigheidscollege te Leuven. Vervolgens koos zij in 2004 om de studie “Logopedische en Audiologische Wetenschappen” te volgen aan de Katholieke Universiteit te Leuven. Tijdens het masterjaar specialiseerde ze zich in de “Audiologische Wetenschappen”. Voor haar masterthesis bestudeerde ze de relatie tussen *auditory steady-state responses* op stimuli aangeboden boven de gehoordrempel en het spraakverstaan. Dit onderzoek werd bij zowel normaalhorende als slechthorende volwassenen uitgevoerd. Haar klinische en wetenschappelijke stage liep ze op de afdeling Keel-, Neus- en Oorheelkunde (KNO) van het Universitair Medisch Centrum te Groningen (UMCG). Daar deed ze onderzoek naar het waarnemen van toonhoogteverschillen door patiënten met een cochleair implantaat.

Na het behalen van haar mastertitel startte ze in 2008 een promotieonderzoek aan de afdeling KNO van het UMCG. Het promotieonderzoek handelde over responsen in het brein van tinnituspatiënten opgemeten met behulp van functionele *Magnetic Resonance Imaging* (MRI). Daarnaast werd er onderzoek gedaan naar structurele veranderingen in de hersenen die geassocieerd zijn met gehoorverlies en tinnitus. Dit onderzoek leidde tot het verdedigen van haar proefschrift en het behalen van de doctorstitel in 2013.

Aansluitend is zij als postdoc gestart op de KNO-afdeling van het UMCG waar zij onderzoek doet naar *gap*-detectie bij tinnituspatiënten. Daarnaast heeft zij een docentenpositie aan de Hanzehogeschool te Groningen geaccepteerd waar zij aan de opleiding “Logopedie” de vakken geluidsleer en audiologie onderwijst en wetenschappelijke ondersteuning biedt. Verder is ze als gastdocent audiologie aan de slag gegaan bij de opleiding “Logopedie” aangeboden door Windesheim Flevoland te Almere.

“Δέδυκε μεν α σελάννα και
Πληιάδες· μέσαι δε
νύκτες, παρά δ' έρχετ' ώρα,
εγώ δε μόνα κατεύδω..”

“The moon has left the sky,
Lost is the Pleiades' light.
It is midnight, and time slips by,
But on my couch alone I lie.”

Sappho (ca. 630-570 B.C.)

Translation: J. A. Symonds, 1883.